CHROMOSOME DAMAGE BY ENVIRONMENTAL AGENTS

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Dedicated to my parents

P.V. Krishna Reddy
P. Sreedevamma

- P.P. Reddy

FOREWORD

During the last two decades, man is exposed to a variety of chemical compounds in the environment. It is estimated that more than 1000 new chemicals are manufactured and marketed in the world annually. These chemicals although are highly beneficial to man to lead a better and comfortable life, they are likely to cause health hazards to man. It is well established that some of the chemicals are found to be mutagenic in different test systems and hence there is every reason to believe or suspect that they are also mutagenic in man. Man is most vulnerable and any undue to exposure to environmental agents might cause chromosome damage in man which in turn increases genetic load. Thus it is obligatory on the part of the scientists to identify these agents and remove them from environment wherever possible. The book compiles the research results of Indian scientists in the field of genetic toxicology. I have no doubt that this book will be useful to the young and enthusiastic research workers in this discipline. I appreciate the pains taking efforts of Prof. P.P. Reddy, in bringing out this volume.

Sd/-
Prof. T. Navaneeth Rao
Chairman, Governing Body,
Institute of Genetics and Vice-Chancellor,
Osmania University.

Place : Hyderabad
Date : 15-8-1989

PREFACE

There is a wide spread concern that exposure of human population to chemicals and environmental pollutants could lead to gradual accumulation of deleterious mutations in the human gene pool. In addition to the consequences to future generations arising from changes in germ cells, there is an increased risk of cancer in the exposed population. During the last few decades there is growing awareness that certain man-made chemicals and some naturally occurring compounds are hazardous to human health. It is important to evaluate the mutagenic and carcinogenic potential of the environmental agents so that appropriate preventive measures can be taken to reduce the genetic load on human population due to environmental pollution. This volume contains articles on the test systems that are applied for identification of environmental carcinogens and other genotoxic agents. This book has been brought out with the objective of disseminating current information on the subject. It is hoped that this publication would stimulate ideas in the young research workers on the theoretical and practical aspects in the area of environmental mutagenesis.

I would like to express my sincere thanks to all the authors who have promptly responded and contributed fine articles. We are highly thankful to Prof. T. Navaneeth Rao, Chairman, Governing Body of the Institute of Genetics and Vice-Chancellor, Osmania University for readily agreeing to write foreword for this book. Thanks are also due to Dr. A. Shobha Rani, Dr. P. Rita and Dr. M. Swarna for their editorial help in the preparation of the book. I am happy to dedicate this book to my parents as a token of my respect. I am thankful to M/s. Indotronix Computers Private Limited, Secunderabad for their help and co-operation in bringing out this volume.

Prof. P.P.Reddy,
Editor.

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Evaluation of mutagenicity of sulfonamides with Ames test.

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Summary

The sulfonamide drugs were the first chemotherapeutic agents to be employed systematically for the prevention and cure of bacterial infections in man (Goodman and Gilman 1971). The mutagenicity of sulfonamides such as sulfamethoxypyridazine, sulfamethoxazole-trimethoprim and sulfaphenazole was tested with S. typhimurium strains TA_{98} and TA_{100} with and without S_9 fraction. Three concentrations of each drug (25,50 and 100 ug/plate of sulfamethoxypyridazine; 10,25 and 50 ug/plate of sulfamethoxazole-trimethoprim; 10,25 and 50 ug/plate of sulfaphenazole) were assayed with TA_{98} and TA_{100} strains directly (without S_9 fraction) and also by incorporating mammalian metabolic activation with S_9 fraction. For each experiment a positive control with Benzo(a)pyrene and a solvent control with DMSO were maintained simultaneously. The results indicate that sulfamethoxypyridazine, sulfamethoxazole-trimethoprim and sulfaphenazole did not increase the frequency of reverse mutations in the strains of S.typhimurium with and without S_9 fraction when compared to controls.

Introduction

The Salmonella microsomal mutagenicity test was used extensively for screening a variety of chemicals for their genetic activity (Ames et al 1975, Maron and Ames 1983). 300 chemicals were tested for their mutagenicity using Salmonella microsome test (McCann et al 1975, McCann and Ames 1976). Among the drugs tested, the results on sulfa drugs were controversial. In view of the above, in the present study, the mutagenicity of sulfamethoxypyridazine, sulfamethoxazole-trimethoprim and sulfaphenazole was tested in TA_{98} and TA_{100} strains of S.typhimurium with or without S_9 fractions.

Materials and methods

L-histidine requiring bacteria of S.typhimurium strains TA_{98} and TA_{100} were used to test the mutagenicity of sulfamethoxypyridazine, sulfamethoxazole-tri-

TABLE 1 AMES MUTAGENICITY TEST IN S.TYPHIMURIUM TA_{98} AND TA_{100} OF SULFONAMIDES WITH S_9 AND WITHOUT S_9 FRACTION.

Test Compound	Concentration tested/ plate	Number of revertants/plate			
		TA_{98}		TA_{100}	
		$+S_9$	$-S_9$	$+S_9$	$-S_9$
Solvent control (DMSO)	100 ul	35	31	171	117.6
Positive control					
Benzo (a) pyrene	2.5 ug	273	267.3	2076.3	2065.3
Sulfamethoxypyridazine	25 ug	21	25	108	126
	50 ug	28.3	26	132	129.3
	100 ug	27.3	30.6	143	136
Sulfamethoxazole - tri	10 ug	39.3	21.6	132.6	134.6
methoprim.	25 ug	43	28.3	136.6	113
	50 ug	41.83	23.6	138.3	133.3
Sulfaphenazole	10 ug	21.5	27	118	125.3
	25 ug	22.3	28.3	108	109.6
	50 ug	19.3	30	143.3	140.3

methoprim and sulfaphenazole. Benzo(a)pyrene, Nicotinamide Adenine-Dinucleotide Phosphate (NADP), Glucose 6-phosphate (Sigma, U.S.A.), L-histidine, biotine (Difco, U.S.A.) were used in these studies. Salmonella typhimurium strains Ta_{98} and TA_{100} were provided by Dr. B.N. Ames (Biochemistry Department, University of California, Berkeley, U.S.A.). All strains were routinely checked for different requirements and characteristics as per the method given by Ames et al (1975) and Maron and Ames (1983).

Preparation of S_9 fraction and mix

Preparation of the liver S_9 fraction was based on the procedure of Garner et al (1972). Aroclor 1254 was diluted in DMSO to a concentration of 200 mg/ml and a single i.p. injection of 500 mg/kg was administered to each rat for 5 days before they were killed by decapitation. Livers were collected and homogenized in 0.15M KCl. The homogenate was centrifuged for 10 mts. at 9000 g. The supernatant was decanted and filtered. It was distributed into sterilized glass screw cap vials and kept on dry ice to freeze the liver homogenate quickly. S_9 mix was prepared according to the recipe recommended by Maron and Ames (1983). 0.1 ml per plate of S_9 mix was used in the experiments.

Mutagenicity test using plate incorporation assays and S. typhimurium strains of TA_{98} and TA_{100} were carried out as described by Ames et al (1975) and Maron and Ames (1983). In mutagenesis assays, the following were added in sequence to 2 ml of melted top agar, 0.1 ml of an overnight broth culture and the test compound or control mutagen. The contents were mixed gently and poured on to the minimal medium plates and distributed uniformly. To another set of the plates, the same contents and 0.2 ml of S_9 mix were added to the minimal medium plates and distributed uniformly. All these plates were inverted and incubated at 37°C. The plates for spontaneous reversions did not contain any chemical. After 48 h incubation the revertant colonies on the test plates and on the control plates were scored in the light background lawn of bacterial growth. The experiments were carried out in triplicate.

Results and Discussion

The results on the effect of sulfamethoxypyridazine, sulfamethoxazole-trimethoprim and sulfaphenazole on TA_{98} and TA_{100} strains of S.typhimurium were given in Table I. Benzo(a) pyrene induced a significant 3 fold increase of revertant colonies in TA_{98} and TA_{100} with and without S_9 fraction. Three concentrations for each drug (25,50 and 100 ug/plate of sulfamethoxypyridazine, 10,25 and 50 ug/plate of sulfamethoxazole-trimethoprim, 10,25 and 50 ug/plate of sulfaphenazole)

were assayed with TA_{98} and TA_{100} strains with and without metabolic activation. The experiments were repeated so as to confirm the results obtained in the first experiment and also to avoid any errors in conducting the study.

The three drugs, sulfamethoxypyridazine, sulfamethoxazole-trimethoprim and sulfaphenazole did not increase the number of reverse mutant colonies per plate in TA_{98} and TA_{100} with and without S_9 fraction when compared to controls. Similar findings were reported by McCann et al (1975) on the effect of sulfaguanedine in Ames test in all Salmonella strains tested. Trimethoprim was reported to have induced reversions only in TA_{98} and TA_{1538} strains with and without S_9 fraction with concentrations of 5 to 15 mg/plate where as in other strains i.e. TA_{100} and TA_{102} it was found to be negative (Rasool et al 1987). In the present study sulfamethoxazole-trimethoprim failed to induce reversions in TA_{98}.

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Plants as environmental pollution indicators

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Summary

Industrilization is beneficial to humanity, at the same time they are polluting the environment through releasing some gases and effluents from the factories. Some of these gases are toxic to plants and biotic life including human beings. It is now believed that some plant species are so sensitive to pollution that they can be used as indicators or monitors of presence of certain kind of pollutants. Hence a study was undertaken to identify such indicator plants around the area of Balanagar where the major industries are drug manufacturing and chemical units, and the parameters investigated were compared with the non polluted area. Due to the air pollution, defoliation, leaf chlorosis, necrosis, bronzing and withering were observed in several species of plants under survey. Out of the fifty one plant species surveyed they were classified as 3 indicators, 12 susceptible, and 26 resistant species based on their sensitivity to pollutants. The indicators were Annona squamosa, Ixora coccinia and Lausonia alba. Hence it is concluded that these plants can be used as environmental monitors.

Introduction

The serious problem which mankind is facing today is that of pollution. By definition air pollution implies the presence of substances above the permissible limit in the air which are injurious to humans. CO_2,CO, SO_2, etc., are some air pollutants. The main sources of pollutants are thermal power stations, fertilizers, factories, textile mills and traffic. It is now becoming clear that certain plant species can be utilized as indicators for atmospheric pollution. Atmospheric pollutants effect the plants in various ways and many reports are available on this subject (Babrov 1955, Middelleton 1961, Jafri et al 1979, Chakrabarthy and Gupta 1981).

This investigation deals with the response of plants to air pollution in relation to the growth and plant metabolism. It would be of very great help in evolving the methods of plant protection, selection or breeding the resistant species and

determining the proper use and planning of land in the industrial area (Heck et al 1973).

It is known that some plant species and varieties are so sensitive to pollutants that they can be used as biological indicators or monitors. For eg. Nicotiana and Asclepias indicate the presence of oxidant pollutants (Middelleton 1961). Corn, gladiolus and tulip indicate the presence of hydrogen flouride (Duchelle et al 1980). Denaeyer (1975) suggested the use of Lolium and Trifolium as bio-indicators in the study of ethylene pollution. It is necessary to the plants for each particular ommission type rather than locating the common plant indicators for all types of areas. Studies on these lines are lacking and hence the present study was taken up with a view to identify pollution indicator plants in industrial areas.

Material and methods

Survey area selected for our investigation

Balanagar industrial area of Hyderabad was selected for our study where the industries are drug manufacturing companies. SO_2, NO_2 and particulates are the main pollutants of this area. The annual averages of the pollutant levels of this area are 12 ug/m^3/24h SO_2, 6ug/m^3/24h NO_2, 245 ug/m^3/24h particulates pH of the water in dust fall in this area ranges from 6.1 to 7.7 (NEERI Hyderabad Report). The annual averages of temperature (maximum and minumum), relative humidity (morning and noon)and rainfall are 32.7°C, 21.2°C, 66%, 43% and 51 cm respectively (Indian Meteriological Department, Begumpet Hyderabad).

Survey

Fortnightly visits were made to Balanagar industrial area for two years, 1984-1986, the vegetation was surveyed and leaf and petiole samples were collected for studying their morphological changes. Vegetation of the residential area (RRL, Colony) was also surveyed for comparison. Fifty one angiosperm species were surveyed and these include 17 herbs, 9 shrubs, and 25 trees. Leaf area and petiole lengths were measured for 29 species in three different seasons (winter, summer and monsoon).

Results and discussion

Except for a few species canopy and foliage of the vegetation of industrial area were comparable to that of their counter parts in residential area. A.squamosa, C. nucifera, Citrus species I. coccinia, L. alba and M. indica from industrial area

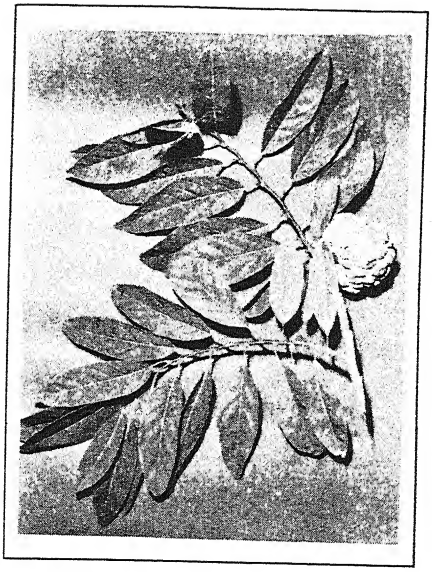

Fig. 1a. Annona squamosa from non polluted area.

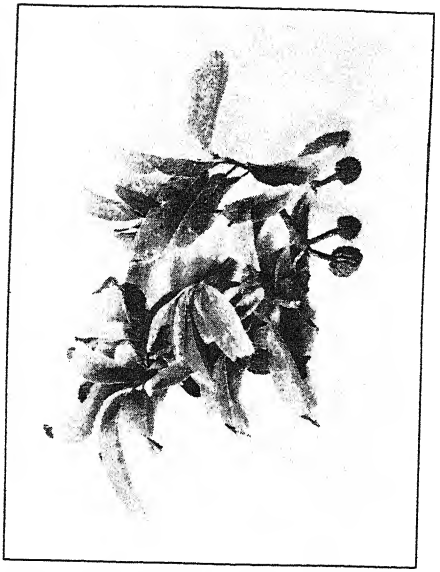

Fig. 1b. Annona squamosa from polluted area showing reduction in leaf size, fruit size, chlorosis and leaf scorching.

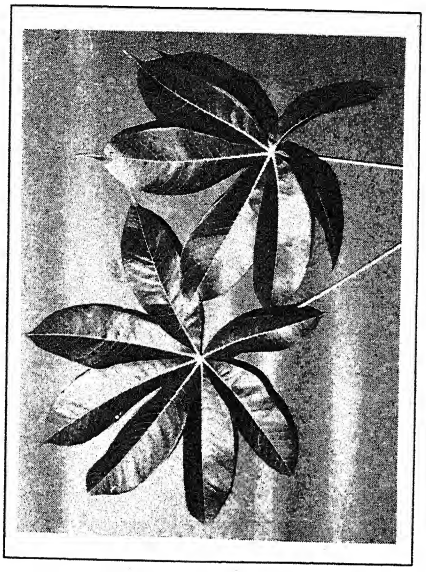

Fig . 2a. Bombax malabarica from non polluted area.

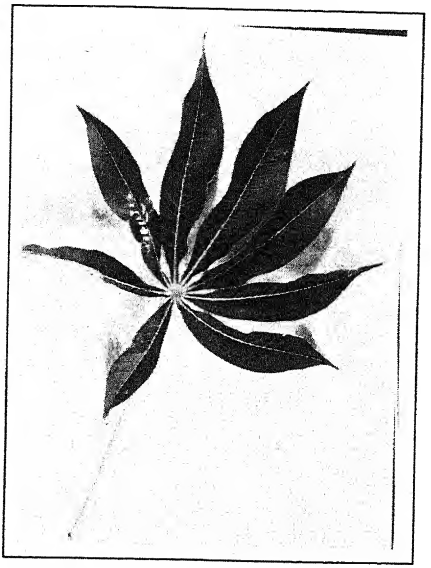

Fig. 2b. Bombax malabarica from polluted area showing leaf mottling.

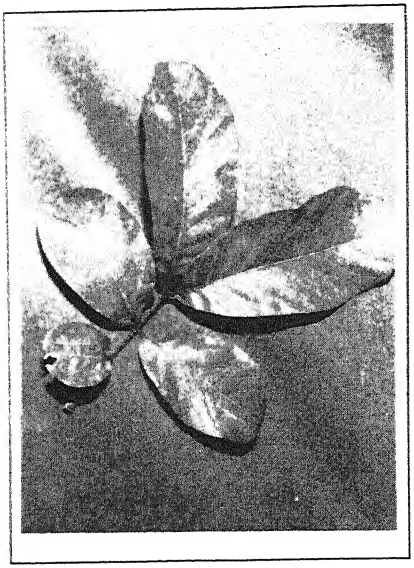

Fig. 3a. Sapindus sapindifolia from non polluted area.

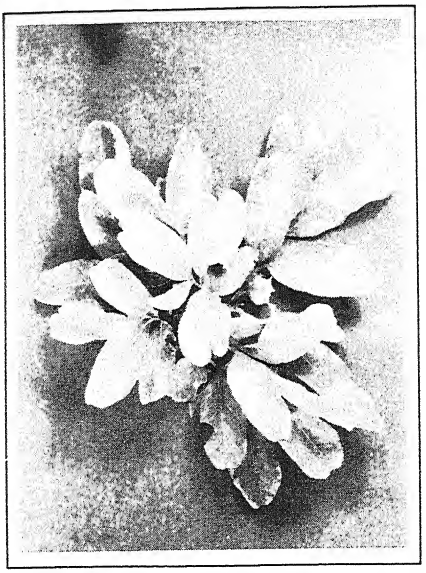

Fig. 3b. Sapindus sapindifolia from polluted area showing reduction in leaf size, chlorosis and leaf burnt.

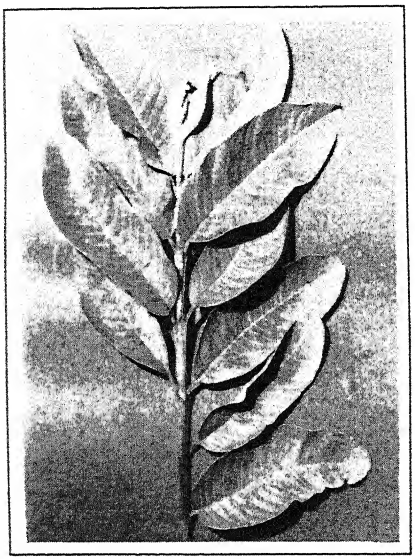

Fig. 4a. Psidium guajava from non polluted area.

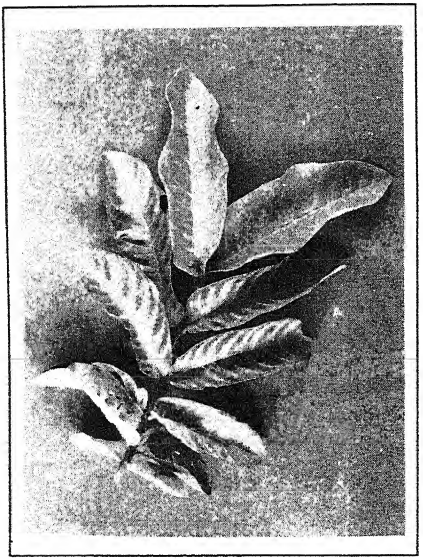

Fig. 4b. Psidium guajava from polluted area showing leaf scorching.

showed reduced canopy and foliage. Defoliation was observed in many of the species in industrial area. Complete defoliation was noted in some of the evergreen trees. C. lemon, C. limonium, P. guajava and S. cumini. Leaf chlorosis, necrosis, bronzing, withering, hyponasty, epinasty, and tip burning were common for majority of the shurbs and trees in industrial area (Figs 1a, b, 2a, b, 3a, b, and 4a, b). However the extent of these symptoms and the time of occurence differed from species to species. Chlorosis pattern differed from species to species. It was in the form of spots in A. indica, C. rosea, Citrus sps, H. integrifolia, L. camara, M. indica, and S.cumini, intravenal in P. alba, marginal in S. sapindifolia and complete in A.squamosa and L. alba. Except in a few species, the leaf area and petiole length of the plants from polluted area were reduced when compared to that of the normal vegetation.

Flowering and fruiting were very much reduced in A. squamosa, Citrus sps, C. nucifera, M. indica, P. guajava, S. sapindifolia, S. cumini of industrial area. Depending on the extent and frequency of morphological variations and the reproductive capacity of the plants in industrial area they are classified into indicator, resistant and susceptible species. Indicators represent the plants with complete impairment of reproductive capacity and recurrence of defoliation and acute morphological deformations. Susceptible species also manifest the symptoms of indicators but to a lesser extent and the resistant species are the ones with normal morphological and reproductive capacity. Of the 49 species surveyed 3 are indicators, 12 are susceptible and 36 are resistant species.

The present study enforces the theory of environmental monitoring by plants. The morphological differences exhibited by the vegetation in the industrial area were species specific and helped for the easy identification of the three classes (indicators, susceptible, and resistant). There is a need for expanding similar studies to many more industries through which urban environment can be monitored.

Acknowledgements

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Excised root culture as a test system for genotoxicity assessment

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Summary

Excised roots in culture are comparable to the roots of the seedlings and both of them can be expected to provide similar response to genotoxic compounds. 2-deoxy-D- glucose (2-DG), a glucose antimetabolite, was reported to alter the incidence of radiation - induced genetic damage in the root meristematic cells of the seedlings of Trigonella foenum-graecum L., an in vivo system. The present study was carried out with a view to examine whether the roots in culture constituting an in vitro system, also exhibit a similar response. Root meristems of T.foenum-graecum were excised and cultured in modified Whites medium (M.W.M). They were irradiated with gamma rays (3 Gy) and immediately exposed to 2-DG for 60 min. Roots not exposed to either radiation or 2-DG constituted the untreated set. Treated roots grown in MWM were fixed and processed to obtain cytological preparations. They were scored to obtain data on the incidence of mitotic anomalies, micronuclei and on mitotic indices. From the observations it is evident that excised roots in culture respond similar to the roots from the seedlings. The results suggest the use of excised roots in culture for genotoxicity assessment.

Introduction

For the assessment of genotoxicity of an agent, it is essential to employ a variety of test systems and to examine several genetic parameters. While laboratory mammals constitute an in vivo system, mammalian cells in culture form an in vitro system for the test. Though cultured mammalian cells have been extensively employed to obtain data on genotoxicity of a variety of agents, plant tissues in culture have not been exploited to the same extent for such an evaluation. This perhaps may be attributed to the tedious technical procedures involved or may be due to the problems encountered in extrapolating the results. Plants are sensitive in situ detectors of atmospheric pollutants (Grant and Zura 1982) and genetic

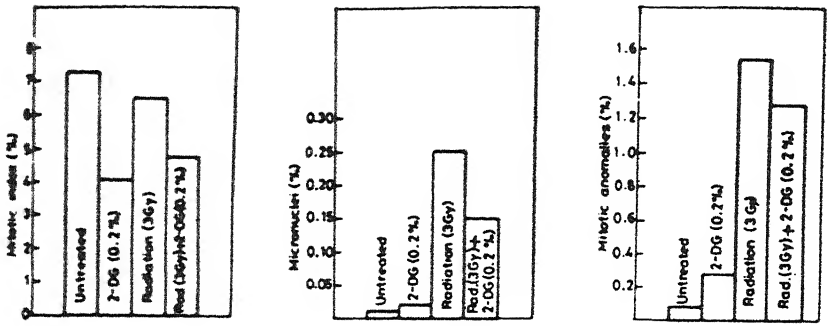

Fig . 1 : Effect of 2-DG on Radiation Induced Cytogenetic Damage in the Cultured Roots of T. foenum-graecum

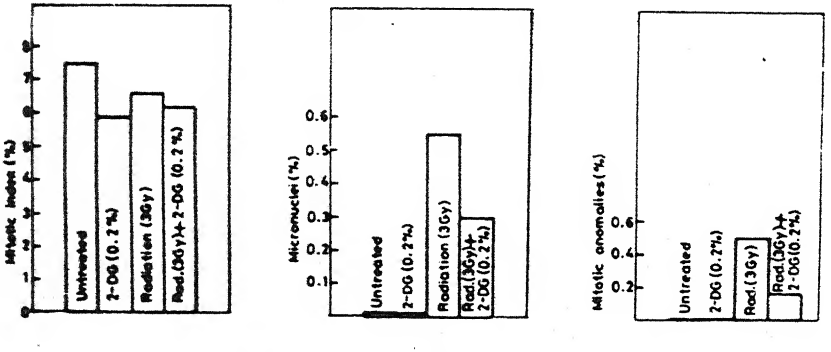

Fig . 2 : Effect of 2-DG on Radiation Induced Cytogenetic Damage in the Root Meristems of T. foenum-graecum (Rao and Gopinath, 1989).

abnormalities induced by various agricultural chemicals in plants are comparable to that observed in animals (Grant 1978, 1982 a,b). Allium test is one of the recommended procedures in the battery of test systems employed for the assessment of chromosomal damage caused by chemicals.

Venketeswaran and Partanen (1966) investigated the cytomorphological effects of gamma radiation on the growth of seedlings and callus cultures of tobacco. Bajaj (1970,1973) studied the effect of gamma radiation on seeds, seedlings and callus cultures of Phaseolus. The survivality and totipotency of haploid tobacco cells in culture exposed to gamma and UV rays were assessed by Eapen (1976). Majumdar and Lane (1970) analysed the effect of sodium cyclamate on Haworthia callus cultures. In vitro activation of chemicals by plants was demonstrated by various investigators (Plewa and Gentile 1982, Plewa et al 1983, 1984 and 1985, Gentile et al 1986). The feasibility of using plant cell cultures for the evaluation of acute and chronic toxicity of chemicals was demonstrated by Turano and Wilson (1985). We have very little information on the effect of genotoxicants in cultured excised roots. An attempt is made therefore to examine the effect of gamma rays and 2-deoxy-D-glucose (2-DG) on excised roots of Trigonella foenum-graecum (Fenugreek) in culture. Results from such a study can be compared with the data on cytogenetic anomalies observed in seedling root meristems with a view to elucidate the utility of cultured excised roots for the evaluation of genotoxicity.

2-Deoxy-D-glucose (2-DG), a glucose antimetabolite is a potent inhibitor of glycolysis and ATP supply (Cramer and Woodward 1952, Woodward and Hudson 1954). 2-DG is reported to differentially act on normal and tumor cells (Jain et al 1977, 1979, Kalia et al 1982). Radiation - induced cytogenetic damage was shown to be modified by 2-DG in the root meristematic cells of T.foenum-graecum, an in vivo system (Rao and Gopinath 1986).

Materials and methods

Seeds were surface sterilized with mercuric chloride (0.1% w/v) for 10 min. and washed thoroughly with sterile distilled water (3 changes) and allowed to germinate in dark in sterile petriplates with cotton and filter paper wetted with sterile distilled water. Cultures were established from root tips excised 24 hr later by inoculating them into modified Whites medium (MWM, pH 4.5) without the supplementation of growth hormones. They were grown at a temperature of 24 $\pm$ 1°C in dark.

Cultured roots were exposed to gamma radiation (3 Gy) and transferred to fresh medium. Another set of irradiated roots were exposed to medium containing 2-DG (0.2%) for 1h and then transferred to fresh medium and incubated. Cultured roots not exposed to either radiation or 2-DG formed the untreated set.

Roots were fixed in ethanol : acetic acid (3:1) and were processed as haematoxylin squashes (Marimuthu and Subramaniam 1960). Slides were made permanent employing tertiary butyl alcohol.

Results and Discussion

The objective of the present investigation was to examine the feasibility of employing excised roots in culture for genotoxicity assessment. Compared to untreated set, 2-DG (0.2% - 1hr) inhibited mitotic activity. Though there was a slight increase in the frequencies of micronuclei and mitotic anomalies, the difference in the values was not statistically significant. Results are presented in Fig.1. Cultured roots exposed to radiation (3 Gy) exhibited a significant increase in the frequency of micronuclei and mitotic anomalies and showed inhibition of mitotic activity. Cultured roots after exposure to gamma rays when post-treated with 2-DG exhibited a reduction in the frequency of micronuclei and mitotic anomalies, compared to roots exposed to radiation alone. These observations are in agreement with our earlier findings (Fig.2) employing invivo system (Rao and Gopinath, 1986). 2-DG reduced the mitotic activity and was found to be non-clastogenic in the roots of fenugreek seedlings. Post treatment of irradiated roots with 2-DG reduced the frequencies of micronuclei and mitotic anomalies induced by gamma radiation.

Roots from seedlings and excised roots in culture exhibited similar response on exposure to radiation, 2-DG or to a combination of both. Use of cultured roots is advantageous in that it is possible to control various factors. Excised roots in culture can therefore be considered an ideal test system for the assessment of genotoxicity of various agents.

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Mutagenic potential of sewage environment - An in situ study with the insect Chrotogonus saussurei bol.,

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Summary

Chrotogonus saussurei, a phytophagus insect and a natural inhabitant of Valankulam sewage tank was selected as a bioassay system to monitor the mutagenic potential of sewage environment. A significant increase in the occurrence of meiocytes with karyotoxic symptoms and specific type of aberrations such as bridge, fragment and vagrants and a significant decrease in the chiasma frequency has been observed. The results indicate that apart from its impact on chiasma formation the Valankulam sewage environment has an increased potential of causing chromosomal aberrations. Based on the findings, Chrotogonus saussurei particularly the lower weight group, has been suggested as a suitable bioassay system to monitor the mutagenic potential of contaminated environments.

Introduction

In situ monitoring is considered as an effective approach to screen the environment for mutagens, as it provides information regarding the magnitude of action of mutagens in the presence of modifying environmental factors in the biological test system, normally a natural inhabitant, hence the target organism. A limited number of in situ monitoring projects conducted in the past decade reveal that mutagens are present in the air, water and soil at significant level (Ma and Harris 1985).

In an industrialized city a large number of xenobiotic substances are introduced into the environment and a considerable amount of which finally find their way directly or indirectly into the sewage system. In Coimbatore city (11°N latitude, 77°E longitude and at about 1425 ft above MSL) the sewage has been discharged into irrigation tanks completely untreated. Valankulam, one such irrigation tank is situated on the southern side of Coimbatore city, has been selected as the site of investigation for the present study. Apart from urban sewage,

TABLE 1 FREQUENCY OF MEIOCYTES (EXPRESED IN PERCENTAGE) WITH KARYOTOXIC SYMPTOMS IN CHROTOGONUS SAUSSUREI OF SEWAGE AND POLLUTED ENVIRONMENT

		CONTROL Cells with				SEWAGE Cells with			
Weight group	Weight (mg)	Cells scored	Vag	Brid and Frag	Total	Cells scored	Vag	Brid and Frag	Total
I	70 - 79	485	1.05	0.61	1.65	301	6.64	2.98	9.61**
II	136 - 146	720	0.41	0.83	1.25	715	3.63	1.39	5.17*
Percentage difference comparison between weight group I and II							8 3.01	1.65	8 4.5

Vag =Vagrancy Brid = Bridges Frag = Fragments
** (P < 0.01) *(P < 0.05) S (P < 0.05) (Proportion Test)

the tank also receives waste water from hospitals and untreated effluents from small scale industries and automobile workshops, hence suspected to contain mutagenic agents and nonmutagenic agents having a role in the activity of mutagenic agents. During rainy seasons, flooding of urban runoff causes extensive mixing and spreading of pollutants to the unpolluted area of the tank. The contaminated water of the tank is used by certain section of people inhabiting the bank of the tank for various human needs apart from its usage for irrigation.

The use of contaminated water for various human needs and contaminated soil for the cultivation of vegetables cause great concern and make man one of the target organisms of harmful influences of sewage environment. Therefore, for the present study one of the target organisms, Chrotogonus saussurei, a phytophagus soil grasshopper and a natural inhabitant of the tank, which lays eggs in the sewage contaminated soil, has been selected as an in situ bioassay system in order to assess the mutagenic potential of sewage environment. Two weight groups of insects one in the lower and the other in the higher range have been selected in order to study the net effect of duration of exposure and magnitude of resistance mechanism on the morphology of chromosomes (clastogenic manifestations), movement of chromosomes in the anaphase stage (turbagenic manifestation) and chiasma frequency. Though several types of mutagen induced clastogenic manifestations are known, in this study only aberrations such as bridges and fragments have been analysed.

Materials and methods

The grass hoppers were collected manually during lean month (August) from the exposed areas of the tank where the common grass Cynodon dactylon has densely grown, brought to the laboratory in well aerated tin boxes and kept in suitable vivaria. The insects collected from unpolluted Bharathiar University campus served as controls.

24 male insects 12 each collected from sewage and unpolluted environments were used for the present study. Of the 12 insects from each environment one half represents weight group ranges from 70 to 79 mg and the other from 136 to 146 mg.

The male insects were dissected out in insect Ringer's solution, testes were isolated and fixed in 1 : 3 acetic alcohol for 24 hours and stored in 70% alcohol at 4°C. A few follicles from the proximal and distal regions of the testes were isolated and placed on clean microslide in a few drops of 1.5% acetocarmine.

The hind regions of the follicles containing mature spermatazoa were excised and removed. The remaining follicles were covered with a coverslip and chromosome spreading was achieved by gentle fingertip pressure. The coverslips

TABLE 2 FREQUENCY OF OCCURRENCE OF TURBAGENIC MANIFESTATIONS (EXPRESSED IN PERCENTAGE)

			VAGRANTS					
			% of cells with				Range	No.of Vag per cell
	No.of cells scored	% of cells with vagrants	1 Vag	2 Vag	3 Vag	4 Vag		
Control Weight group I	485	1.05	0.41	0.62	—	—	0 — 2	0.016
Sewage Weight group I	301	6.64	3.32	2.33	0.10	- -	0 — 3	0.11
Control Weight group II	720	0.41	0.28	—	—	0.14	0 — 4	0.008
Sewage Weight group II	715	3.63	1.54	0.98	0.84	0.42	0 — 4	0.077

TABLE 3 FREQUENCY OF OCCURRENCE OF CLASTOGENIC MANIFESTATIONS (EXPRESSED IN PERCENTAGE)

	No of cells scored	% of cells with brid	Range/ cell	No. of brid/ cell	% of cells with Frag	Range/ cell	No. of Frag/ cell
Control Weight group I	485	0.21	0 - 1	0.002	0.41	0 - 1	0.0041
Sewage Weight group I	301	1.33	0 - 1	0.013	1.66	0 - 1	0.017
Control Weight group II	720	0.42	0 - 1	0.00417	0.417	0 - 1	0.0042
Sewage Weight group II	715	0.42	0 - 1	0.00419	0.98	0 - 1	0.0098

Brid = Bridge Frag = Fragment

TABLE 4 FREQUENCY OF CHIASMATA IN THE MEIOTIC CELLS OF CHROTOGONUS SAUSSUREI

Weight group	CONTROL POLLUTION				SEWAGE POLLUTION			
	Frequency of Loop/ cell	Ring/ cell	Rod/ cell	Chiasma frequency/ cell	Loop/ cell	Ring/ cell	Rod/ cell	Chiasma frequency/ cell
I	0.783	5.56	2.66	16.13	0.33	3.87	4.80	13.53**
II	0.92	5.75	2.33	16.58	0.51	4.29	4.19	14.31**
Mean				16.36				13.92
				± 0.22				± 0.38

** ($P < 0.01$)

were wax ringed and slides were examined for the various end points of toxicity. The slides were made permanent following the procedure of Darlington and LaCour (1976) and representative slides were photographed.

For testing significance of variance student 't' test and proportion test were applied.

Results

Data on the frequency of meiocytes with karyotoxic symptoms in two weight groups of insects collected from sewage and unpolluted environments are presented in table I. In both the weight groups of insects collected from the sewage environment, the frequency of meiocytes with karyotoxic symptoms (table 1) which includes turbagenic and clastogenic manifestations, shows a significant increase, when compared to the same in the respective weight groups of insecis collected from the unpolluted environment.

In the weight group I, the frequency of meiocytes with turbagenic manifestation is 6.64% and clastogenic manifestation 2.98%, whereas in the control the frequency of the former is 1.05% and the latter 0.61%. The increase in the frequency of meiocytes with turbagenic manifestations is statistically significant.

In the weight group II also a statistically significant increase in the frequency of meiocytes with turbagenic manifestation (3.63%) and clastogenic manifestation (1.39%) is observed when compared to the respective frequency value of 0.41% and 0.83% observed in the control. Further, when such manifestations are compared within the sewage population between two weight groups, a reduction to the tune of 3.01% in the turbagenic manifestation and 1.65% in the clastogenic manifestation and therefore in the total karytoxic symptoms (4.5%)is observed.

Since data on frequency of meiocytes with karyotoxic symptoms do not provide information such as number or range of specific type of aberration per cell, further analysis of affected cells have been carried out and various details concerned with turbagenic and clastogenic manifestations are presented in the Table 2 and 3.

In the weight group I, the range of occurrence of vagrants per cell (rpc), percentage of cells with different number of vagrants (pdv) and number of vagrants per cell (nvpc) show an increase over the respective values observed in the control group of insects. In the weight group II, even though maximum number of vagrants increased to 4 in both population of insects, the percentage increase in the pdv and nvpc is less when compared to the respective values in the control. However, when such data are compared between the weight group I and weight group II, a significant decrease with increase in weight is observed in the values of pdv and nvpc.

A detail analysis of clastogenic manifestation reveals that sewage environment does not have an inductory effect on the formation of bridges in the higher weight group, eventhough, such an effect is noticed in the lower weight group of insects. However, appreciable impact of sewage environment on the formation of fragments is noticed in both the weight groups of insects but in the descending order of magnitude.

Data on chiasma frequency per cell in the control and in the sewage population are presented in Table 4. The chiasma frequency in both the weight groups of insects (13.53 and 14.31) show a significant decrease when compared to the control (16.13 and 16.58).

The mean chiasma frequency of sewage population taking into account both weight groups is 13.92 ± 0.38 whereas in the control the observed value is 16.36 $\pm$ 0.22. The reduction in the chiasma frequency in the sewage population is statistically significant.

Discussion

The increase in the frequency of meiocytes with clastogenic and turbagenic manifestations and in the percentage occurrence of specific aberrations such as bridges, fragments and vagrants in the sewage population when compared to the respective values observed in the control insects clearly indicate the occurence of mutagenic agents in the sewage environment of Valankulam. Occurrence of such aberrations in the control insects collected from the unpolluted environment may be attributed to spontaneous aberrations. Occurrence of spontaneous aberrations have been reported in several organisms (Yamaghuchi and Mukai 1974 , Yamaghuchi et al 1976, Abraham 1965, Demerick 1937, Brusick 1980).

The observed decrease in the percentage occurrence of clastogenic and turbagenic manifestation in the weight group II when compared to the weight group I in the sewage population reveals that lower weight group of insects are more susceptable to the action of mutagenic agents than the higher weight group. Effective functioning of acquired mechanisms which minimize the action of hazardous anthropogenic agents (mutagens) on the hereditary material and / or the increased role played by repair mechanisms may be the reasons for the decrease in the clastogenic and turbagenic manifestations in the higher weight group. Occurrence of chromosomal aberrations in the insects from unpolluted environment indicates that agents other than anthropogenic in origin may be responsible for the same. The increase in the frequency of aberrations in the sewage population may be the result of direct action of anthropogenic agents on the hereditary material or decrease in the effectiveness of inherent resistance mechanisms due to the action of anthropogenic agents thereby exposing the hereditary material to the

increased action of natural intrinsic or extrinsic mutagenic agents. The possibility of synergistic or additive actions of anthropogenic agents with natural mutagenic agents as a cause for the increase in aberration frequency also cannot be ruled out.

Chiasma frequency characterizes pairing of homologus chromosomes at meiosis and controls the degree of recombinations besides influencing fertility. It provides the organism a mechanism for adaptation to new habitats (Sun and Rees 1964). Mutagenic agents have been reported either to increase (Bennet and Rees 1970) or decrease (Prasad and Godward 1969, Goud 1967, Sree Ramulu 1971, Sinha and Roy 1976, Sadanandam and Subash 1984) the chiasma frequency. In few cases (Singh and Mahapatra 1969, Jain and Basak 1965) no reduction in chiasma frequency has been observed. The reduction in the chiasma frequency in both the weight group of insects collected from Valankulam sewage environment, when compared to the control is in agreement with the findings of Sree Ramulu (1971) in Sorghum, Singh et al (1977), Lal and Srinivasachar (1979) in Pearl Millet and Sinha and Roy (1976) in Phaseolus. The reduction in the chiasma frequency as suggested by Sadanandam and Subash (1984), may be attributable to the nature and potency of mutagens present in the sewage environment and also to underlying factors such as complex structural change or due to mutagen induced change in the nature of genes responsible for chiasma formation. The observed reduction in the number of loops representing 3 chiasmata and rings 2 chiasmata with a concomitant increase in the frequency of rods (1 chiasma) may be due to rapid terminalization of chiasmata (Sree Ramulu 1971) or decrease in the crossing over due to the action of mutagenic agents.

The ubiquitous distribution, the occurrence of large sized and small number of chromosomes (18 autosomes and X chromosome) and the result of the present investigation clearly indicates that Chrotogonus saussurei particularly the lower weight is more suitable as a bioassay system for the in situ monitoring of mutagenic potential of contaminated environments.

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PABA induced genotoxic effects in silkworm Bombyx mori

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Summary

Paraminobenzoic acid (PABA) is a bacterial vitamin and an essential component of artificial food for insects in general and for silkworms in particular. It is needed in small quantities for normal growth of silkworms. Hence the genotoxic effect of high concentrations of PABA was studied, using an indigenous bivoltine race Kalimpong-A of silkworm Bombyx mori. 1, 2 and 3 g/50 worms of the chemical was administered by topical application technique. The chemical could not induce dominant lethals at the different concentrations tested. However, fertility, viability and hatchability were reduced significantly, while the larval duration was increased in all the concentrations tested. Hence PABA though non mutagenic, is toxic to silkworms at high concentrations.

Introduction

Chemical growth regulators are extensively used to improve yield in plants (Singh et al 1978, Raza 1978, Nelson and Sharples 1980, Agarwal 1984) and animals (Akai 1971, Akai and Kobayashi 1971, Kobari and Akai 1978, 1979, Krishnaswami et al 1979, Pai and Krishnamurthy 1984, Pai et al 1987). Paraaminobenzoic acid (PABA) is one such essential growth regulator present in minute quantities (0.02ppm) in artificial food and tissue culture medium for silkworm Bombyx mori. Since there is a dirth of information on PABA induced genotoxic effects in silkworm B.mori, an attempt has been made to study the PABA induced genotoxic effects in silkworm B.mori.

Materials and methods

Indigenous bivoltine race Kalimpong - A maintained for over 25 generations at germ plasm bank of Sericulture Research Project, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, India formed the material for the present study. Two hundred healthy larvae of approximately equal

weight and size were selected on 1st day of V instar and were divided into four groups of 50 worms each. The PABA was administered following the topical application technique (Tazima 1978). 1g/50 worms, 2g/50 worms and 3g/50 worms of the chemical was dissolved in distilled water and painted evenly on dorsal and ventral surfaces of the larvae. The larvae smeared with distilled water alone were taken as control. The larvae were allowed to continue the development with mulberry leaves as feed upto spinning. Further, the moths obtained from the above groups were crossed with moths of opposite sex obtained from the same groups and were allowed to lay eggs on egg sheets. These eggs were also allowed to develop and hatch into young larvae. When hatching was completed larval duration, viability, fecundity, fertility and hatchability were estimated. The results obtained therein were subjected to statistical analysis by Anova test.

Results

Table 1 incorporates the data on the effect of PABA on larval duration, viability, fecundity, fertility and hatchability in Kalimpong-A race. The data reveal that the treatment of PABA significantly ($P< 0.05$) prolongs the larval duration from 576.0 ± 5hr. in control to 605.0 ± 5hr. in 2g and 3g of PABA/50 worm concentrations, while at the concentration of 1g of PABA/ 50 worms the larval duration did not alter significantly. Further viability reduced significantly from $93.00 \pm 1.5\%$ in control to $60.00 \pm 2.5\%$, $59.00 \pm 3.6\%$ and $57.00 \pm 3.2\%$ at 1g, 2g and 3g of PABA/50 worms concentrations respectively. Fecundity was significantly lowered ($P< 0.05$) from 537.0 ± 13.0 in control to 462.0 ± 11.5 and 456.0 ± 16.0 in 2g and 3g of PABA/50 worms. However in the concentration of 1g of PABA/ 50 worms did not induce significant variation with regard to fecundity. Further, the treatment of all the three concentrations of PABA significantly reduced the fertility. Hatchability also was reduced significantly in the groups of silkworms treated with 2g of PABA/50 worms ($76.46 \pm 4.64\%$) and 3g of PABA/ 50 worms ($68.00 \pm 2.46\%$) compared to control ($95.50 \pm 1.16\%$).

Discussion

Rate of development, viability, fecundity, fertility and hatchability are some of the important parameters to evaluate toxicity of a chemical in animal test system (Hanna and Dyer 1973, Jayasuriya and Ratnayake 1973, Laamanen et al 1976). These serve as good indicators of various somatic effects caused by a chemical in the test substrate (Lunning 1966, Sorsa and Pfeifer 1973 a, b). Therefore the genotoxicity of various environmental agents is assessed using these parameters.

TABLE 1 PABA INDUCED GENOTOXIC EFFECTS IN SILKWORmBOMBYX MORI

Mode of Administraion : Topical application.
Race Used : Bivoltine Kalimpong - A (Average of 10 layings)

	Control	Concentration 1. (1g/50 worms)	Concentration 2. 2g/50 worms)	Concentration 3. (3g/50 worms)
Fecundity	537.0 ± 13.0	514.0 ± 12.5	462.0 ± 11.50$	456.0 ± 16.00$
Fertility (%)	97.0 ± 0.88	90.10 ± 1.16$	83.36 ± 3.26$	76.62 ± 2.26$
Viability (%)	93.00 ± 1.50	60.00 ± 2.50$	59.00 ± 3.60$	57.00 ± 3.20$
Hatchability (%)	95.50 ± 1.16	92.00 3.11	76.46 ± 4.64$	68.00 ± 2.46$
Larval duration in hours	576.0 ± 5.00	600.0 ± 5.0	605.0 ± 5.0$	605.0 ± 5.0$

The present study showed that PABA is toxic in silkworm. The growth regulators have the potentiality to either decrease or increase the lifespan of organism. Akai et al (1978) and Kuwano et al (1984) could prolong larval duration in silkworm using juvenile hormone and manta respectively. Viability is an adaptive trait of any individual to survive and perpetuate in a given environment. In the present investigation the viability was found to decline significantly indicating that PABA has profound effect on viability in silkworms.

Fecundity is one of the fitness component of an organism. The reduced fecundity is not favoured by natural selection, which eliminates the genotype in due course. In the present experiments fecundity was found to be reduced from 537.0 ± 13.0 in control to 462.0 ± 11.5 and 456.0 ± 16.0 in 2g and 3g of PABA/ 50 worms. However in the concentration of 1g of PABA/ 50worms fecundity did not show a significant variation from the control. The reduction in fecundity might be due to changes in hormonal system due to chemical treatment. But Gruwez et al (1971) reported that the number of eggs produced may depend on number of ovarioles present in the organism. Activity and number of ovarioles inturn depend on the conditions in which the larvae have been submitted. The experiment reveals that PABA brings in a significant ($P<0.05$) dose dependent reduction in fertility in silkworms. The fertility reduced from 97.05 ± 0.88% in controls to 90.10 ± 1.16%, 83.36 ± 3.26% and 76.62 ± 2.26% in the concentrations of 1g, 2g and 3g of PABA/50 worms respectively. Hatchability is another important parameter to study the toxicity of a substance in any test system. Reduction in hatchability is attributed to the effects of the chemical on growing embryo (Sankaranarayanan 1969, Vasudev and Krishnamurthy 1979, Rajasekarasetty et al 1981). In the present investigation the groups of silkworms treated with 2g of PABA/ 50 worms and 3g of PABA/ 50 worms showed significant reduction in hatchability from 95.50 ± 1.16% in control to 76.46 ± 4.64% and 68.00 ± 2.46% respectively, indicating that PABA affects hatchability significantly at high doses. But at low concentrations PABA does not affect hatchability. Thus the results of these studies show that PABA is toxic to silkworm B.mori in the above concentrations.

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IAA induced ontogenic variation in acid and alkaline phosphatase in silkworm Bombyx Mori

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Summary

Indole acetic acid (IAA), a plant growth regulator is being extensively used in plants and animals to improve yield. It increases the yield either by enhancing or inducing the cellular synthesis. The ontogenic changes in pure Mysore race induced by IAA was studied using polyacrylamide gel electrophoretic techniques. 200ppm of growth regulator was administered to the silkworm race by egg treatment and oral feeding methods. 11 acid and 11 alkaline phosphatase zones were observed in groups obtained by egg treatment method in comparison with 13 acid and 10 alkaline phosphatase zones in control. Further, 13 Aph and 9 Acph zones were observed in orally fed groups in comparison with 11 Aph and 10 Acph zones in control, indicating ontogenic variation between control and treated groups.

Introduction

Indole acetic acid (IAA) is a plant growth regulator, which is being used extensively to improve the yield in plants. It is also being used for improving the yield in mulberry, the leaves of which are being used as feed for silkworm Bombyx mori. There is no information on the genetic and toxicological effects of IAA in silkworms. Further, the gel electrophoresis is a precise and efficient technique, that is being presently used for studying genetic structure of natural populations (Lewontine and Hubby 1966, Ayala et al 1972,1974). This technique is also being used to study the ontogenic variability and differentiation since it permits precise detection of electrophoretic variants of different proteins including isozymes (Yoshitake 1966, Eguchi et al 1965, Hegde and Krishnamurthy 1980). This promted the authors to evaluate the IAA induced ontogenic variation in alkaline phosphatase (Aph) and acid phosphatase (Acph) in silkworms by using gel electrophoretic technique.

Materials and methods

Pure Mysore, a multivoltine race of silkworm B.mori available at Sericulture Research Project, Department of Studies in Zoology University of Mysore, Manasagangotri, Mysore-570 006 formed the material for the present investigation. The chemical IAA was administered into the test system by i) Egg treatment and ii) Oral administration methods.

i) Egg treatment method: Healthy male and female moths emerged out of cocoons were allowed to mate for 3h. Then they were depaired and the female moths were allowed to lay eggs on egg sheets. As soon as the eggs were laid, eggs of same age (± 4h.) were subjected to IAA treatment by soaking them in 200 ppm of IAA in distilled water for 30 minutes. The chemical gets into the egg through the porous shell. Later the eggs were washed in distilled water and were allowed to develop further. The eggs soaked in distilled water for 30 minutes served as control. After 24h, 40mg of eggs were separately collected from control and treated groups and were homogenised with 2 ml of distilled water. The remaining eggs were allowed to continue their development. The whole body homogenate of same concentration was prepared at I instar, II instar, III instar, IV instar, V instar larval stages, male pupa, female pupa, male moth and female moth stages and these homogenates were subjected for polyacrylamide gel electrophoretic techniques (Davis 1964). After electrophoresis, the gels were stained for Aph and Acph using histochemical stains (Hegde 1979) and the zymograms were made.

ii) Oral administration: The healthy silkworm larvae were reared upto II moult with normal mulberry leaves as feed. As soon as they enter the III instar stage, 100 healthy larvae were reared separately by feeding them with the leaves soaked in 200 ppm of IAA and were later dried in a cool and dry place. The treatment was continued till spinning stage. The control were fed with mulberry leaves soaked in distilled water, which were later air dried. In this experiment also worms of III instar, IV instar, V instar larvae, male pupa, female pupa, male moths and female moths were subjected for electrophoresis and zymograms were prepared on the basis of electrophoretic mobility of the enzymes, which were represented in the form of bands on the gels.

Results

The zymogram pattern for Aph and Acph at different developmental stages of untreated and IAA treated Pure Mysore race of silkworm Bombyx mori are presented in Figures 1 to 6. First column of each zymogram indicates a composite picture of isozyme pattern throughout the life cycle, while remaining columns denote zymogram pattern observed at any given stage of development. Differential staining capacity of the bands are also presented in the zymogram.

Composite zymogram for Aph in untreated (Control) Pure Mysore race shows 13 bands. However, none of the developmental stages exhibit the presence of all the 13 Aph bands. A maximum of 4 bands were observed at V instar, while egg, pupa and moths of both sexes showed 2 Aph bands each with variable electrophoretic mobility.

Fig.2 gives the zymogram pattern obtained for Acph in control, which showed a maximum of 3 bands at III instar stage and a minimum of 1 band at egg and female moth stage. A total of 10 Acph bands were observed in composite zymogram of all the developmental stages.

Zymogram obtained for Aph from egg treatment groups is presented in fig.3. Aph activity is reduced from 13 bands in control to 11 bands in treated groups. Further, IAA treatment reduced Aph activity in egg, II instar, V instar and moths of both sexes, while there is an increased enzyme activity in I instar and III instar.

Fig. 4 depicts IAA induced ontogenic variation in acid phosphatase in Pure Mysore race of silkworm B.mori. The zymogram shows an increased Acph activity from 10 bands in control to 11 bands in treated. Further IV instar, V instar and Pupal stages of treated groups showed dark bands in comparison with light bands in untreated. In addition, a maximum of 4 Acph bands were observed in II instar of treated groups in comparison with a maximum of 3 Acph bands in same stage of control.

Fig. 5 shows the IAA induced variation when administered orally to pure Mysore race. The zymogram shows a reduction in Aph activity from 10 bands in control to 9 bands in treated groups. The treatment also affects the electrophoretic mobility.

Acid phosphatase activity is found to increase from 11 bands in control to 13 bands in orally administered groups of silkworm B.mori (Fig. 6). Further as in Aph activity, electrophoretic mobility is altered in the treated groups.

Discussion

Fecundity, fertility, viability, rate of development etc., are parameters that are generally used to evaluate the toxicity of any chemical or physical agent. The

Fig. 1 The Pattern of ontogenic variation of alkaline phosphatase in pure Mysore race of silk-worm BOMBYX MORI

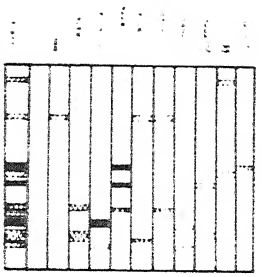

Fig. 2 The zymogram pattern of ontogenic in pure Mysore race of silkworm BOMBYX MORI

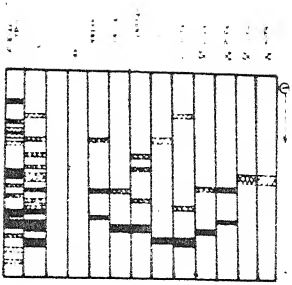

Fig. 3 The zymogram pattern of IAA induced ontogenic variation of alkaline phosphatase activity in pure mysore race of silkworm BOMBYX MORI (Egg treatment)

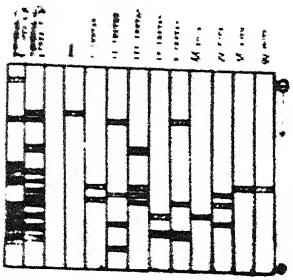

Fig. 4 T. ymogram pattern of IAA induced ontogenic variation of acid phosphatase activity in pure Mysore race of silkworm BOMBYX MORI (Egg treatment)

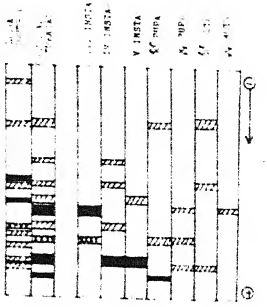

Fig. 5 The zymogram pattern of IAA induced ontogenic variation of alkaline phosphatase activity in pure Mysore race of silkworm BOMBYX MORI (Oral administration)

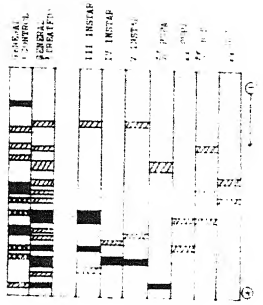

Fig. 6 The zymogram patterns of IAA induced ontogenic variation of acid phosphatase activity in pure Mysore race of silkworm BOMBYX MORI (Oral administration)

Dark Moderate Faint

toxic or mutagenic effect of a chemical could be attributed to the genetic damage in the form of chromosomal abnormalities or due to point mutations including alteration in nucleotide sequence at molecular level resulting in the change of gene action. The electrophoretic technique which generally used to detect the alteration in the amino acids of proteins can be conveniently be used to detect these changes.

In the present studies the authors have studied the effect of IAA on the expression of alkaline and acid phosphatase in the multivoltine race of silkworm B.mori. The administration of this growth regulator has brought about immense changes in the electrophoretic patterns of these enzymes. In some instances, the treatment enhances the enzyme activity while in others it decreases the activity. For eg., the treatment of IAA increases the number of Acph bands from 10 in control to 11 in treated (fig.4) and 11 in control to 13 in treated (fig. 6), when administered by egg treatment and oral administration methods respectively. However the number of Aph bands were reduced in the treated groups. In some instances the electrophoretic mobilities of different bands are altered. The expression of the isozyme forms depend on the physiological state of the cells (Markert and Ursprung 1962). Perhaps the treatment of growth regulators alter the physiological state of cells and hence the expression of isozymes forms vary.

The electrophoretic variation in the treated groups also depends on the route of administration. For eg., the oral administration of IAA increases the number of Aph bands from 10 in control to 9 in treated, while the administration by egg treatment method decreases the number of Aph bands from 10 in control to 9 in treated. Kuwano et al (1967), Sugiyama et al (1969), Sato et al (1975) and Sugiyama (1980) have shown that route of administration of the chemical has profound effect in a given test system. The present studies also reveal that route of administration of the chemical has immense effect on the expression of enzymatic activity in silkworm Bombyx mori. Thus the results confirm that the electrophoretic technique, in addition to its use in molecular, population and developmental genetic studies can conveniently be used for genotoxic studies.

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Genetic control of mosquitoes: mating competitiveness of translocation heterozygote males of Anopheles Stephensi liston— a malaria vector in laboratory cage trails.

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Summary

To assess the practical application of using chromosomal translocations in the genetic control programme of Anopheles stephensi, mating competitive tests were carried out using semisterile males with male-linked translocation T(Y:2L)1 with normal females in the presence of normal males in laboratory condition proved that translocated males compete 1.93 percent higher than normal males.

Introduction

A large number of radiation induced chromosomal translocations have been isolated in Anopheles stephensi Liston, one of the important carriers of malaria in Indian subcontinent. These include both sex-linked and autosomal. Each translocation heterozygote showed characteristic sterility with little variation.

The development of chromosomal translocations for the genetic control of the mosquito, An.stephensi, has progressed to a point where evaluation of the mating competitiveness of translocation males is necessary. This mating competitiveness is usually first tested experimentally under insectary conditions using laboratory adapted strains. Estimation of mating competitiveness of a Y-autosomal translocation heterozygote by laboratory tests conducted by Terewedow et al (1972) was the method used here in the present investigation. Similar reports of tests conducted in the laboratory and in the field in a few species of mosquitoes have also appeared in literature.

Materials and methods

A laboratory strain of An.stephensi, ARC pureline was used to study the mating competitiveness of the translocated line. The translocation line used for this purpose was T(Y:2L)1 heterozygote. This is one of the most interesting and

promising translocations. All the females dissected showed normal karyotypes, whereas all the dissected males revealed unequal exchange between the longer autosome and short arm of YL. The break point on 2L is at 20D. This translocation which is inherited only through males was considered suitable for a field release study. The strain is characterized by 56.52 percent sterility in males and 6.5 percent sterility in females. This line has passed 34 generations and expansion of this stock is very simple. It was maintained in the laboratory by outcrossing each generation to the females of ARC pureline.

Virgin males and females were used for all the crossing experiments. Three types of experiments (two controls and one competition) were conducted simultaneously, each in triplicate. They are as follows: A normal control of 50 ARC♂s and 50 ARC♀ s. A translocation control of 50 T(Y:2L)1♂ s and 50 ARC♀ s and competition test with 100 T(Y:2L)1♂ s, 100 ARC♂s and 100 ARC♀ s.

The competitiveness of the translocated males was calculated from hatchability data, using the formula of Haisch (1970).

$$e = \frac{(q - f)}{n\,(f - p)}$$

Where: q = Proportion of hatched eggs in the normal control which consisted of wild-type females mated with males of their own strain; f = Proportion of hatched eggs from the competition studies; p = Proportion of hatched eggs of the translocated control; n = ratio of T(Y:2L)1♂ to ARC♂.

Results

Control : The data of crossing experiments of the normal control has been represented in Table-1.

Set - 1 : 24 females laid eggs and the percent hatch rate was 79.23%.
Set - 2 : Out of 50 females, 38 laid eggs and the percent hatch rate was 86.72%.
Set - 3 : 34 females laid eggs and the percent hatch rate was 79.60%.
On an average 96 females, out of 150, showed a percent hatch rate of 81.85%.

Translocation control: The data of the translocation control has been represented in Table-1.
Set - 1 : 25 females laid eggs and the percent hatch rate was 34.87%.
Set - 2 : 33 females laid eggs and the percent hatch rate was 42.76%.

Set - 3 : Out of 50 females 26 laid eggs and the percent hatch rate was 44.57%.
On the average 84 females, out of 150, showed a percent hatch rate 40.73%.
Competition The average data on the competitiveness of translocated males T(Y:2L)1 has been represented in Table-1.
Set - 1 : Out of 100 females, 64 females laid eggs and percent hatch rate was 49.46%.
Set - 2 : 89 females laid eggs, out of 100 females and the hatch rate was 59.41%.
Set - 3 : Out of 100 females 78 laid eggs and the hatch rate was 55.32%.
On an average, out of 300 females 231 females laid eggs and percent of egg hatches was 54.73%.

The competitiveness was

$$e = \frac{81.85 - 54.73}{54.73 - 40.73}$$

$$e = 1.93\%$$

TABLE 1 ANOPHELES STEPHENSI COMPETITION STUDIES IN SMALL LABORATORY CAGES INVOLVING : T(Y:2L)1 TRANSLOCATION AND A LABORATORY STRAIN ARC.

Sl. No.	Strain	No. of batches	Total eggs	Total larvae	% egg hatch
1.	ARC	24	3,091	2,449	79.23
2.	ARC	38	4,172	3,618	86.72
3.	ARC	34	3,810	3,033	79.60
1.	T(Y:2L)1	25	2,294	800	34.87
2.	T(Y:2L)1	33	3,556	1,478	42.76
3.	T(Y:2L)1	26	2,818	1,256	44.57
1.	ARC vs T(Y:2L)1	64	6,316	3,124	49.46
2.	ARC vs T(Y:2L)1	89	9,656	5,737	59.41
3.	ARC vs T(Y:2L)1	78	8,136	4,501	55.32

Discussion

As indicated earlier, one of the sex-linked chromosomal translocations, T(Y:2L)1, was selected to test the possibility of using such mechanisms in the control of An.stephensi. In order to verify the mating competitive ability of the translocated males, laboratory cage experiments were designed and conducted. The mating competitive ability of the translocated males were calculated by using the formula of Haisch (1970). It was found that in each cage the translocated males competed higher than the normal males. Though a slight variation between these cages was apparent these differences were not significant. The average value (mating competitive ability) of the three cages was calculated and estimated to be 1.93%. These observations confirmed the fact that genetically engineered mosquitoes succeed in small cages under constant laboratory conditions. Moreover, the translocations were isolated from the pureline. The results clearly show that the sex-linked translocation described here offers an excellent material for conducting genetic experiments involved in the suppression of An.stephensi. Future objectives include experiments under field conditions. If the mating competitive ability of the translocated males proves to be higher than that of normal males, field trials will be conducted.

Mating competitive tests in laboratory conditions involving genetically altered males have been reported in different species of mosquitoes. Males of An.culicifacies carrying a complex chromosomal rearrangement were released in a small field experiment to test their mating competitiveness. In this experiment, the laboratory males were found to be equally competitive with the endemic males for marked laboratory-adapted and laboratory-reared wild virgin females. In another study utilizing the genetic sexing mechanisms, males sterilized with bisazir were less competitive in the field although equally competitive under laboratory conditions (Baker et al 1982).

In Culex tarsalis, Terewedow et al (1977) have shown that the competitive studies in laboratory and field cages demonstrated that the translocated males were capable of mating competitively with different colony strains. However, in the pilot release experiments the translocated males were unsuccessful in mating competition with the natural populations (Asman et al 1979). In Culex guinguefasciatus the mating competitive ability of the sex-linked double translocation heterozygotes were higher than the wild males both in the laboratory and field conditions. Leven (1969) observed in Culex pipiens that males with the Y-linked translocation compete higher than the wild males in the laboratory. Later this strain was released into the field where the translocated males competed higher than the native wild males.

In Culex triteaniorhynchus, Baker et al (1982) observed that the chromosomally altered males competed higher than the wild males both in the laboratory and in the field conditions. Similar observations were also recorded in Aedes aegypti (Grover et al 1976, Sea Wright et al 1976 and 1977).

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Studies on the frequency of spermatozoa with abnormal heads of mice treated with possible ester derivatives of para-amino benzoic acid

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Summary

The mutagenic activity of four metabolites of para-amino-benzoic acid has been tested in mice and all the metabolites were found to induce sperm head abnormalites in mice.

Introduction

Para amino benzoic acid plays an important role as vitamin in bacteria and it is included in artificial foods as an essential constituent for insects particularly silk worm (Pai et al 1988). It is widely used in sun-screen and other topical preparations (Gichner et al 1987, Gichner and Veleminsky 1988) and hence, the populations at large are exposed to this chemical. It is because of this, it has been subjected to various mutagenic assays wherein it has yielded contradictory results. In some it acts as a mutagen, while in others it antagonises the toxicity caused by some nitroso compounds. It is because of this controversy the present studies were undertaken with the possible metabolites of this compound so that some light can be thrown on its mechanism of action as a mutagen as well as an anti-mutagen. Although studies have been carried out on sister chromatid exchanges, micronuclei, dominant lethals and the abnormalities in the heads of spermatozoa, this report communicates the results obtained in the last test system only. It has been suggested that the frequency of abnormal heads of spermatozoa is under genetic control (Bruce and Heddle 1979) therefore this assay may be used for assessing the mutagenicity of physical and chemical agents in germ cells (Wyrobek and Bruce 1975, Soares 1979, Topham 1980, Sobti et al 1987 a, b and 1988).

Material and methods

Male mice (5 in each group) of Lacca strain obtained from the Central Research Institute, Kasauli (H.P.) were given six injections of 0.1 ml of 10^{-3}M/kg

chemical on alternate days. The smears of spermatozoa were made from the epididymis after 5 weeks of the last exposure of the chemical (Wyrobek and Bruce 1975). The slides were stained in Giemsa and studied for the abnormalities in the heads of spermatozoa. Parallel controls were maintained for each chemical.

Results and Discussion

All the four metabolites studied elevated the frequency of spermatozoa with abnormal heads. Their frequency with different chemicals alongwith the relative incidences are given in Table-1. The relative percentage incidence ranged from 58.30 (methyl-p-aminobenzoate) to 140 (Methyl-Para-nitrobenzoate). In the latter, the frequency of abnormalities is 7.20 ± 0.56.

TABLE - 1 FREQUENCY OF ABNORMALITIES IN THE HEAD OF SPERMATOZOA INDUCED BY METABOLITES OF PABA

Chemical	Dose	Duration	Mean±SD	Relative incidence
Methyl p-aminobenzoate	10^{-3}M/kgx 6	"	4.75 ± 0.35*	58.30 $p < 0.05$
Methyl p-nitrobenzoate	10^{-3}M/kg x 6	"	7.20 ± 0.56**	140.00 $p < 0.01$
Methyl p-nitrosobenzoate	10^{-3}M/kg x 6	"	3.10 ± 0.80	70.00 $p < 0.01$
O-Acetyl-N-acetyl 4 Carboxy methyl-phenyl Hydroxylamine	10^{-3}M/kg x 6	5 weeks	5.45 ± 0.33**	67.60
Control			3.00 ± 0.14	**$p < 0.01$

On analyzing the data, it is apparent that methyl-p-nitrobenzoate produced highest number of sperms with abnormal heads, the values with methyl-p-nitroso benzoate, Nacetyl-O-acetyl 4-carboxy methyl phenyl hydroxylamine stood almost equal. The frequency of spermatozoa with abnormal heads produced by methyl-p-nitrobenzoate was 7.2 ± 0.56, the value being much higher than those with other compounds indicating, that what ever mutations were caused by this

compounds were capable of crossing the barrier of testes and blood much more frequently than other compounds. When these results were compared with the results on the other test systems used by the present workers, interesting conclusions are apparent, especially with regard to the dominant lethal test. Though nitrosobenzoate caused lesser abnormalities in the heads of spermatozoa, the mutations were probably so lethal that they did not allow any impregnation in the females. The lethality of the mutations caused by methyl-p-aminobenzoate was also evident. Infact, when the results on micronuclei, dominant lethal and the frequency of spermatozoa with abnormal heads were compared, the net result was that, although p-nitroso benzoate produced less number of micronuclei and less number of spermatozoa with abnormal heads, the ultimate lethality towards embryos was maximum indicating therein maximum genotoxicity of this compound, proving, the contention put forth by Gupta et al (1987) and Sobti et al (1987a) with regard to the high mutagenicity of nitroso over nitro compounds. The former authors had worked on Salmonella typhimurium, while the results of latter were based on experiments with mice. It has been put forth that nitro compounds on metabolic activation change into nitroso i.e., $NO_2 = NO$ which in turn changes to NHOH. There is no doubt that the pathway is from nitro to nitroso and then to hydroxylamine, but more reactions are leading towards detoxification when it reaches to amino derivatives. It is quite evident from the current observations on micronuclei (unpublished data) that the relative incidence with nitro is 40%, whereas with nitroso it is 100% and as soon as it reaches to hydroxyl and amino, it comes down to 70%. This is probably the reason that PABA has been found to be antimutagenic in many of the test systems worked out by various authors.

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Genotoxicity of malachite-green in Swiss male mice

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Summary

The genotoxicity of malachite green was tested in Swiss male mice. It was found to be genotoxic at concentrations of 5, 2.5 and 1.25mg/kg in mice.

Introduction

Malachite green is extensively used all over the world for dying silk, wool, jute fabrics and leather. It is also commonly used in laboratories for staining bacterial spores and histopathological sections. Though malachite green is legally banned in food industries, it is still commonly used in rural areas for colouring food stuffs. This paper deals with the genetic toxicity of malachite green using sperm shape abnormality assay. The results indicated that malachite green is genotoxic in mice.

Materials and methods

Inbred Swiss albino-mice were used for experiments maintained under standard conditions. A test substance malachite-green (CAS registry number-569-64-2) was obtained from E. Merck (India). The sterile solution of malachite-green in distilled water (D.W.) was injected intraperitoneally (i.p.) to male mice of eight weeks old. For each dose three animals were employed and equal number of negative controls were used. The negative controls were injected with sterile distilled water (D.W.). Experimental procedure reported by Wyrobek and Bruce (1975) was employed. After treatment, animals were divided into two groups and housed in separate cages. One group of animals were sacrificed after seven days and the second group thirty five days after the first injection. Mice were killed by cervical dislocation and smears of sperms were made as described by Krazanowska (1976). A total of 2000 sperms from each animal was scored for head shape abnormalities at 450X magnification. Different sperm head abnormalities were scored as per the recommendation of Wyrobek and Bruce (1975). Percentage of abnormally shaped sperms was the parameter used in assessing genetic toxicity of the test compound.

Results

Results obtained are given in Table 1, indicating dosages used and percent abnormal sperms observed seven and thirty-five days after treatment.

TABLE 1 EFFECT OF MALACHITE GREEN ON SPERM HEAD ABNORMALITY IN MICE

++ Test substance	+++ Dosage mg/kg	Percent abnormal sperms ±SE	
		Seven days after the treatment	Thirty five days after the treatment
Negative control	D.W.	2.57 ± 0.09	2.90 ± 0.08
Malachite green	5.0	6.75 ± 1.11θ	7.2 ± 1.31θ
	2.5	5.87 ± 1.5θ	6.02 ± 1.43θ
	1.25	4.75 ± 0.64θ	5.2 ± 1.03θ

+ 2000 sperms examined for each mice
++ Three mice were kept for each dose
+++ Intraperitoneal administration

Discussion

From the above Table, it is clear that, malachite-green is capable of inducing significant increase in sperm head abnormalities both seven and thirty-five days after treatment at dosages of 5, 2.5. and 1.25 mg/kg. Sperms observed seven and thirty five days after the treatment were in sperm and spermatogonial stage respectively (Wyrobek and Bruce 1975). It is therefore, clear that both sperm and spermatogonia are affected after the treatment with Malachite-green indicating that it is capable of crossing the testes barrier. There is evidence that sperm head abnormality is the consequence of damage in genetic material (Wyrobek and Bruce 1975, Soares et al 1979, Topham 1980).

The clastogenic ability of malachite-green is also reported by Godbole (1980) using in vivo micronucleus assay and analysis of chromosome aberrations

from bone marrow cells of mice. Muller and Gautier (1975) have also shown that malachite-green has a capacity of binding to DNA of different microorganisms in AT rich regions. Brenner et al (1958) have shown that basic dyes are capable of reacting with acidic group of DNA molecule. It is therefore possible that a basic dye, malachite-green might be reacting with acid group of DNA molecule to bring about genotoxic activity and consequently increasing the percent of abnormally shaped sperm heads.

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Screening of some pesticides for mutagenic activity in mice

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Summary

Phorate and monocrotophos are well known contact and systemic insecticides and cholinesterase inhibitors. Contradictory reports on their mutagenic potential prompted us to monitor their genotoxic effect. In the present study the micronucleus test, sperm head assay and chromosomal aberrations in spermatogonia of mice have been used to assess the mutagenic activity of phorate and monocrotophos in comparison to mitomycin C, a well known potent mutagen. The results show phorate to be slightly mutagenic in micronucleus test and non-mutagenic in the other two tests. Monocrotophos did not differ significantly from the results shown by the negative control. Mitomycin-C was strongly positive in the three tests used for the study.

Introduction

Pesticides are an essential component of modern civilisation for they are a great help in reducing losses in agricultural sector due to insect pests besides decreasing the annual loss of human health and life caused by insect borne diseases. But despite the economic and disease control benefits resulting from pesticide use, humans sometimes pay a heavy price in terms of health and environment problems. The pesticidal mutagenic agents constitute a prime area of human concern as several pesticides have been shown to possess mutagenic properties (Waters et al 1980). Thus determination of the mutagenic potential of the myriad of pesticides that are encountered in the environment is of utmost importance for safeguarding human health.

Conflicting data available on the genotoxic effect of phorate and monocrotophos prompted us to monitor their mutagenic potential. The present study deals with three in vivo tests, the chromosomal aberration in spermatogonia, micronucleus test and sperm head assay in mice.

Materials and methods

Male albino mice of Swiss inbred strain (Mus musculus) weighing 23-25 g. were procured from the Biological Evans Private Limited, Hyderabad. The two

TABLE 1 FREQUENCYOF MICRONUCLEI INDUCTION FOLLOWING ORAL TREATMENT IN MICE WITH MITOMYCIN C, MONOCROTOPHOS AND PHORATE

Dosage* mg/kg body weight	Number of mice	Number of Polychromatic erythrocytes (PCE)	Number of PCE with micronucleus	Percentage of PCE with micronucleus	Number of normochromatic erythrocytes (NCE)	Number of NCE with micronucleus	Percentage of NCE with micronucleus
1. Control	5	10,000	26	0.26	10,402	9	0.08
2. Mitomycin C							
3.187	5	10,000	270	2.7**	10,901	20	0.178
3. Monocrotophos							
3.53	5	10,000	33	0.33	11,408	12	0.11
7.06	5	10,000	38	0.38	10,600	12	0.11
10.59	5	10,000	36	0.36	12,868	13	0.12
4. Phorate							
0.38	5	10,000	49	0.49	10,314	15	0.14
0.76	5	10,000	65	0.65	10,502	19	0.18
1.14	5	10,000	82	0.82***	10,606	25	0.23

* Administered in 2 equal halves

** P<0.01 *** P < 0.05

Differences in frequencies were statistically analysed by student `t' test.

TABLE 2 THE EFFECT OF MITOMYCIN C, MONOCROTOPHOS AND PHORATE ON THE FREQUENCY OF ABNORMAL SPERMS IN MICE EXPOSED ORALLY

Dosages* mg/kg body weight scored	Number of mice	Total number of sperms	Normal sperms		Abnormal sperms	
			Number	Percentage	Number	Percentage
1. Control	5	5000	4914	98.28	86	1.7
2. Mitomycin C						
3.187	5	5000	4898	97.96	251	5.1**
3. Monocrotophos						
3.53	5	5000	4882	97.64	118	2.36
7.06	5	5000	4902	98.04	98	1.96
10.59	5	5000	4894	97.88	106	2.12
4. Phorate						
0.38	5	5000	4898	97.96	102	2.08
0.76	5	5000	4889	97.78	111	2.22
1.14	5	5000	4880	97.06	120	2.4

* Administered in 5 equal fractions

** $P < 0.05$

Differences in frequencies were statistically analysed by using student `t' test

organophosphates, i.e., phorate (Volrho) O,O-diethyl S-ethyl thiomethyl phosphorodithioate - Technical grade having 70% purity with CAS Registry number (298-02) and monocrotophos (NOCIL) (Cis-1-methyl-2-methyl carbamoyl vinyl phosphate - Technical grade having 71% purity with CAS Registry number (6923-22-4) were tested along with a known mutagen mitomycin C (Sigma). The doses were selected on the basis of LD_{50}. Five animals per dose were incubated with the test chemicals. The volume of pesticide solution administered was 0.5ml. The control animals received a similar volume of the vehicle.

The method of Schmid (1975) was followed for micronucleus test. The sperm head assay was performed according to the method of Wyrobeck and Bruce (1975) with some minor alterations, that is, all the animals were sacrificed thirty five days after treatment and procedure of Evans et al (1964) was adhered to chromosomal aberrations in spermatogonia. The results were statistically analysed by the 't' test and X^2 test.

Results

Micronucleus test: Table 1 summarises the results obtained from the induction of micronuclei in the bone marrow of mice. There was a gradual increase in percentage of micronuclei from 0.26 in control to 0.82 at the highest dose level of phorate, 1.14 mg/kg. Statistical analysis of the data showed significant incidence of micronuclei only at the highest dose level in the phorate. The percentage of polychromatic erythrocytes with micronuclei showed no significant difference in the micronuclei between the control and monocrotophos treated mice. In sharp contrast there was a drastic increase in the frequency of micronuclei in mitomycin-C treated mice as compared to the control. The analysis of results showed significant differences in the incidence of micronuclei between control and mitomycin-C treated mice.

Sperm head assay : Phorate and monocrotophos treated mice showed no significant increase in the frequency of abnormal sperms in any of the treated groups as shown in Table 2, whereas the frequency of aberrant sperms increased significantly in mitomycin-C treated group.

Studies on chromosomal aberrations in spermatogonia :The results showed no increase in the frequency of aberrations in meiotic cells as compared to control in phorate and monocrotophos treated mice as shown in Table 3. Consequently, the statistical analysis of the data showed no significance. On the other hand mitomycin-C induced significant number of chromosomal aberrations.

TABLE 3 INDUCTION OF CHROMOSOMAL ABERRATIONS IN SPERMATOGONIA OF MICE EXPOSED ORALLY TO MITOMYCIN C, MONOCROTOPHOS AND PHORATE

Dosage* mg/kg body weight	Number of mice	Total number of meta-phases screened	Number of nor-mal me-ta phases	Number of ab-normal meta-phases	Type of abnormal metaphase recorded			
					Sex univalents	Autosomal univalents	Aneuploids	Poly-ploids
1. Control	5	500	453	47	24	14	4	5
2. Mitomycin C								
3.187	5	500	428	72**	26	23	4	19
3. Monocrotophos								
3.53	5	500	457	43	19	16	3	5
7.06	5	500	449	51	22	15	4	10
10.59	5	500	455	45	20	16	4	5
4. Phorate								
0.38	5	500	447	53	22	18	5	8
0.76	5	500	450	50	19	16	8	7
1.14	5	500	442	58	24	19	5	10

* Administered in 5 equal fractions

** $P < 0.05$

Differences in the frequencies were statistically analysed by using 2 x 2 contingency X^2 formula

Discussion

A literature survey has revealed conflicting results on the genotoxic effects of phorate and monocrotophos. The hither-to published data find phorate non-mutagenic in all bacterial and some plant and animal test systems (Gentile et al 1982, Jeang et al 1978, Waters et al 1980, Hsiu et al 1984) and mutagenic in some plant and animal assays (Singh et al 1977 and 1979, Sobti et al 1982). Similarly, contradictory reports of the mutagenicity of monocrotophos have been obtained from literature (Waters et al, 1980, Hanna and Dyer, 1975, Klopman et al, 1985, Dean, 1972 and Panda and Sharma, 1979). The discrepancy between the various test systems could be because it is most unlikely that all the significant aspects of mammalian activation and inactivation can be duplicated in an in vitro assay. Certainly, problems of absorption, excretion and distribution of chemicals are not significant in the in vitro tests. The negative results obtained in the present investigations could be due to the fact that organophosphorus insecticides are rapidly broken down and eliminated.

Pesticides are indispensable in modern agriculture and human health programme but there is a need to regulate and limit the pesticides in use to the recommended levels in the environment.

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A study on the mutagenicity of Bordeaux mixture, an antifungal agent using micronucleus test

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Summary

The genotoxic effect of copper fungicide, Bordeaux mixture was studied employing mouse bone marrow micronucleus test. The concentrations used were, 0.25%, 0.5%, 1.0%, and 2.0% solutions in distilled water. A significant increase of micronucleated cells observed at higher concentrations ($p < 0.05$) indicated the mutagenicity of this compound.

Introduction

Bordeaux mixture is a copper fungicide which is extensively used for the control of fruit rot and bud rot diseases caused by Phytophthora. This compound is effective against other fungal diseases also. In 1885 Millardet and Gayon at the university of Bordeaux, France, first studied the antifungal property of this chemical mixture. Since then this mixture of copper sulphate and lime received wide recognition as antifungal agent (Nene 1971). Bordeaux mixture is nothing but a chemical combination of copper sulphate and quick lime. Millardet and Gayon thought that this reaction proceeded according to the equation,

$$CuS0_4 + Ca(OH)_2 = Cu(OH)_2 + CaS0_4$$

However, the reaction between copper sulphate and lime is rather complex (Nene 1971). Spraying with 1% Bordeaux mixture controls the fruit rot and bud rot diseases satisfactorily. Indiscriminate use of this fungicide is likely to pollute the environment. In the present investigation the mutagenicity of Bordeaux mixture has been studied by employing micronucleus test. This is one of the simplest and cheapest in vivo test system. Since the introduction of micronucleus test by Schmid (1973) various chemicals and drugs have been analysed for their mutagenic potency by using this test (Maier and Schmid 1976, Chaubey et al 1977, Bruce and Heddle 1979, Vaidya and Patanker 1980, Luca et al 1981 Vijayalaxmi 1987).

TABLE 1 MICRONUCLEI (MN) IN THE BONE MARROW ERYTHROCYTES OF MICE TREATED WITH BORDEAUX MIXTURE AND CONTROL ANIMALS.

Treatment	A % PCE	% NCE	% MN in PCE ± SEM	% MN in NCE ± SEM	P/N ± SEM
Control	52.03	47.97	0.21 ± 0.02	0.11 ± 0.019	1.09 ± 0.022
Bordeaux Mixture 0.25%	52.33	47.67	0.20 ± 0.04	0.10 ± 0.02	0.91 ± 0.020
0.5%	51.57	48.43	0.41 ± 0.04*	0.17 ± 0.02	0.94 ± 0.03
1.0%	41.13	58.87	0.65 ± 0.07*	0.18 ± 0.05	0.70 ± 0.03
2.0%	43.26	56.74	1.05 ± 0.13*	0.40 ± 0.11*	0.76 ± 0.02
Endoxan 100mg/kg	30.10	69.90	5.70 ± 0.35*	1.05 ± 0.09*	0.35 ± 0.01

* $P < 0.05$

PCE = Polychromatic erythrocytes

NCE = Normochromatic erythrocytes

SEM = Standard error of mean

A - From 2000 PCE/Animal
5 animals/treatment

Extensive work has been carried out on the genotoxic effects of various pesticides and fungicides using different test parameters (Carere and Morpurgo, 1981, Kappas 1981, Zdzienicka et.al. 1981, 1982, Amer and Abou-ela 1985, Grover and Malhi 1985). However, the genotoxic effect of Bordeaux mixture has not been reported. Guha and Bhattacharya (1986) have reported the cytogenetic effect of copper sulphate, one of the component of Bordeaux mixture in Allium sps. Reduction in mitotic index values and induction of various cytological abnormalities by copper sulphate in green alga, Chara has also been reported (Rumapal and Chatterjee 1987).

Materials and methods

Bordeaux mixture made in distilled water was orally administered to Swiss albino mice (8-10 weeks) in 0.2ml quantity. The different concentrations used were 0.25%, 0.5%, 1.0% and 2.0% solutions. 1% solution is the normally used concentration to control the fungal diseases. The negative control group was administered with identical quantity of dist. water. Mice fed with endoxan (100mg/kg b.w.) formed the positive control group. Bone marrow preparations were made from all the groups at 24 h. using 5% bovine albumin as suspending medium (Seetharam et al 1983). The slides were stained with May-Grunwald-Giemsa. Five animals were used for each treatment and control group. The slides were analysed for the presence of micronuclei (MN) in polychromatic (PCE) and normochromatic erythrocytes (NCE). Statistical significance of the results was assessed by using 't' test.

Results and Discussion

The experimental results are presented in the Table 1. The increase in the micronucleated PCE was statistically significant at concentrations of 0.5%, 1% and 2% while in NCE the increase in MN was significant only at 2%. Slight decrease in the P/N ratio was observed at higher concentrations.

The in vivo micronucleus test is one of the useful bioassays to monitor the genotoxicity of environmental chemicals. Erythrocyte micronucleus represent the consequences of chromosomal aberrations induced during preceeding mitotic division in the erythroblasts. The increase in MN frequency in PCE was dose dependent in Bordeaux mixture treated animals. This is the first report on the mutagenic effect of this chemical mixture in mice bone marrow test system. From the results of present study it may be concluded that, Bordeaux mixture is capable of inducing chromosomal aberrations in mammals. Hence, greater care should be taken while handling or spraying this agricultural chemical.

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Mutagenicity studies of country liquor

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Summary

Arrack(Brandy) sold in licenced shops as well as home brewed liquor from Kerala and local brands of liquor namely mosumbi and santra sold in Maharashtra licenced shops were tested for mutagenicity in Ames test with or without S9 mix. Dose dependent mutagenicity of arrack was observed in strain TA 98 and TA 100 with or without S9 mix. Subsequently, eight different brands of arrack were tested in strain TA 100 at the highest dose (200 ul/plate) and variation in mutagenicity was observed. Dose dependent mutagenicity of mosumbi and santra liquor was observed in TA 98 and mosumbi was found to be negative in TA 100 whereas santra was found to be weakly positive in TA 100. Both the varieties of liquor did not have any further effect with S9 mix.

Frequency of micronuclei increased significantly when mosumbi and santra were tested in micronucleus test, santra being more potent in both the test systems.

Introduction

Excessive intake of alcoholic beverages results in a variety of health hazards e.g. increase in oesophageal (Wynder and Bross 1961), laryngeal (Tunys and Audigier 1976) and oral cancer (Wynder et al 1957). Ethanol consumption during pregnancy causes spontaneous abortions (Olegod et al 1979) and fetal alcohol syndrome (Abel 1984). Ethanol also shows clastogenic effect (ICPEMC Reports, 1987).

In India, low income population consume locally brewed liquor or liquor sold in licenced shops. Since alcohol has been implicated as a risk factor for oesophageal cancer, we have tested three brands of liquor viz. arrack (Kerala brandy) and mosumbi and santra (Maharashtra brands) in Salmonella/ Microsome assay. Mosumbi and santra liquors were also tested in the micronucleus test.

Materials and methods

Arracks (Kerala local brandy) were purchased from local market in Kerala. Santra and mosumbi liquors (Maharashtra local brand) were purchased from local market in Bombay.

Bacterial strain: Salmonella typhimurium strain TA 98 and TA 100 were obtained from Dr.B.Ames, Berkeley, California, USA.

Animals: 8 weeks old Swiss male mice employed in present study were from the animal colony of Cancer Research Institute, Bombay. The animals were maintained on a standard diet. Water and food were supplied ad libitum.

Preparation of S9 mix: The S9 fraction was obtained from Aroclor induced (ip) male Wistar rat liver and S9 mix was prepared according to the method of Ames et al (1975).

Mutagenicity assay: The mutagenicity of arrack, santra and mosumbi liquor were tested in presence and absence of S9 mix by the method of Ames et al(1975). 4-nitro-O-phenylene diamine (NDPA), N-methyl-N'-Nitro-N-nitrosoguanidine (MNNG) (-S9) and Benzo(a) pyrene (B(a)P) (-S9) were used as positive controls. Results are expressed as mean $\pm$ SE of 8 plates.

Micronucleus test: The test was done according to the procedure of Schmid (1975).

Results

Table 1 gives the number of revertants for tester strain TA 100 with one dose of 8 different brands of arrack with or without S9 mix.

Mosumbi was found to be mutagenic in TA 98 and was negative in TA 100, while santra was mutagenic in TA 98 and weakly mutagenic in TA 100. Both the liquors did not show enhanced mutagenicity upon metabolic activation.

In micronucleus test at near LD50 dose mosumbi induced 0.63 $\pm$ 0.04 % of polychromatic erythrocytes (PCE) with micronuclei. P/N ratio was found to be 0.831.

Santra induced 1.13 $\pm$ 0.1 % of PCE with micronuclei and P/N ratio was found to be 0.807.

Distilled water (D/W) treated animals were used as controls having 0.3 $\pm$ 0.03 % of PCE with micronuclei. P/N ratio was found to be 1.05.

Ethylmethane sulfonate was used as positive control having 3.2 $\pm$ 0.04 PCE with micronuclei. P/N ratio was found to be 0.97 at 5 mg/animal dose level.

Results are expressed as mean $\pm$ SE. $P < 0.005$ for mosumbi when compared with D/W treated animals and $P < 0.0005$ for santra when compared with D/W treated animals.

We observed significantly increased number of micronuclei with mosumbi and santra liquor, santra was more potent in this respect.

TABLE 1 MUTAGENICITY OF DIFFERENT BRANDS OF LOCAL ARRACK IN STRAIN TA 100 WITH OR WITHOUT METABOLIC ACTIVATION.

DOSE : 200 u1/plate

Brands	His Revertants per plate	
	-S9	+S9
S.R.	29 ± 6	59 ± 8
1	328 ± 9	542 ± 14
2	235 ± 8	426 ± 10
3	232 ± 14	343 ± 14
4	43 ± 9	643 ± 14
5	330 ± 14	541 ± 14
6	427 ± 9	603 ± 24
7	319 ± 5	627 ± 14
8	507 ± 8	723 ± 34
B(a)P*	-	786 ± 34
MNNG**	798 ± 39	-

Results are mean ± S.E. of 8 plates from two independent experiments.
Spontaneous Revertants are not subtracted.
* Benzo(a)pyrene : Dose - 2 ug/plate
** N-methyl-N'-Nitro -N-Nitrosoguanidine. Dose 2 ug/plate.

Discussion

Our data show that arrack, santra and mosumbi are mutagenic with or without S9 mix in a dose dependent manner. It is pertinent to mention here that Japanese sake, whisky, red wines and beer were found to be mutagenic in TA 100 (Tomita et al 1982). When beer was analysed, volatile nitrosamines were detected (Sen et al 1982). Whiskies, brandies and apple brandy are also reported to have mutagenic potential. Mutagenicity of whisky was demonstrated without S9 mix and was found to be stronger in TA 100 than in TA 98 (Nagao et al 1981). We observed increased frequencies of micronuclei when santra and mosumbi were tested in micronucleus test. Ethanol is shown to have clastogenic effect when it is

metabolised to acetaldehyde (ICPEMC Report, 1987). Matsushima (1987) showed that chronic alcoholics exhibit significantly higher frequencies of chromosomal aberrations in peripheral leukocytes as compared to control group. Tumor promoting activity was noted for Japanese sake when tested in mice initiated by DMBA (Kuratsune et al 1987). In conclusion, we found that arrack, santra and mosumbi were mutagenic in Ames test. Santra and mosumbi increased the frequencies of micronuclei in our studies with both the test systems, santra was found to be more potent than mosumbi.

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Carcinogenicity studies of tobacco - specific N - nitrosamines

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Summary

The tobacco-specific N-nitrosamines (TSNA) have been directly implicate in human cancer. Unburnt tobacco products like chewing tobacco and snuf contain very high amounts of TSNA. Since these nitrosamines are the onl carciongens identified in unburnt tobacco products, a direct relationship of TSN/ to human oral cancer can be assumed.

We have carried out long-term carcinogenicity studies on 'N'-nitrosonorni cotine (NNN) and 4-(methylnitrosamino) -1- (3-pyridyl) -1- butanone (NNK), (th two potent TSNA) using Swiss male mice. The results of these studies show tha both NNN and NNK are strong carcinogens.

Introduction

A correlation between oral cancer and chewing of betel quid (which ofte contains tobacco) and tobacco has been established in India (Sanghvi 1981) an other South-East Asian countries (Winn 1984). The tobacco specific N-nitrosam ines (TSNA) are the only carcinogens identified in unburnt tobacco products TSNA further increase during the process of curing of tobacco (Hoffmann et a 1985, Bhide et al 1987). The exposure of humans to the tobacco-specific N nitrosamines, N'-nitrosonornicotine (NNN) is by far the largest among exposur to nitrosamines (Fine 1978) except for some occupational exposures.

The present study was undertaken in an attempt to induce oral tumors i Swiss male mice and to assess the carcinogenicity of the two important TSNA viz NNN and NNK in Swiss male mice.

Materials and methods

Chemicals : NNN and NNK were synthesized according to the methods o Hu et al (1974) and Hecht et al (1983) respectively.

Animals: Swiss strain mice were obtained from the animal colony of th Cancer Research Institute, Bombay. The animals were housed in metal cages a $21 \pm 1^{\circ}C$. Food and water supplied ad libitum.

Dose: 3-4 hrs prior to treatment 1% atrophine solution was supplied in drinking water to reduce salivation.

1 mg of NNN/NNK (contained in 10 ul of distilled water) was carefully delivered on the ventral side of the tongue twice a week till total dose was delivered. After treatment, the animals were deprived of food and water for one hour in order to retain the nitrosamines in the oral cavity.

1. NNN (Low dose) - A total dose of 22 mg/animal.
2. NNN (high dose) - A total dose of 72 mg/animal.
3. NNK - A total dose of 22 mg/animal.
4. Distilled water - (Solvent) controls.
5. Untreated controls.

The animals were weighed periodically. After the completion of the treatment, the animals were kept under observation and the tissues were fixed in 10% formalin for histological analysis.

Results

The tumorigenic effects of NNN and NNK in Swiss male mice are summarized in Table 1.

The target organs of NNN tumorigenicity were lung, stomach and liver. The lower dose of NNN induced tumors in the lung of 2/8 (25%) animals in the 10-16 months group. However, the same dose induced tumors in 13/19 (68%) animals in the later age group (17-22 months). These included lung adenomas, forestomach papillomas and a hepatocellular adenoma. Apart from these frank tumors, hyperplasia of the basal epithelium of the forestomach was observed in 1/8 animals in the earlier age group (10-16 months) and 3/19 animals in the older age group (17-22 months). The high dose of NNN induced tumors in 10/16 (62.5%) animals in the 10-16 months interval, which included lung adenomas (8/16 animals) and forestomach papillomas (6/16 animals). In the latter age group, the higher dose of NNN induced tumors in 5/10 animals (50%) which included lung adenomas (3/10 animals) forestomach papillomas (2/10 animals) and two liver tumors, out of which one was hepatocelluar adenoma and the other hepatocellular carcinoma. Two out of ten animals showed intense hyperplasia of the basal layer of the forestomach epithelium in the later age group. One animal in the earlier age group showed hyperplasia with excessive keratinization in stomach epithelium.

NNK also induced tumors in the lung, forestomach and liver of the treated animals at both the age groups. The treated animals showed tumors in 9/16 (56%) animals in the earlier age group (10-16 months), which included lung adenomas (8/15 animals), forestomach papillomas (2/15 animals) and a hepatoma. At the

later age group, tumors were induced in 10/13 (77%) of the animals treated with NNK. These included lung adenomas (9/13 animals) forestomach papillomas (1/13 animals) and a hepatoma. The distilled water treated controls did not develop tumors at any site at both the intervals However, lung adenomas were observed in 2/18 (11%) untreated animals at 22 months (Table 1).

TABLE 1 TUMOR INCIDENCE IN SWISS MALE MICE TREATED WITH NNN/NNK

Treatment group	Tumor incidence at 10-16 Months	17-22 Months
Untreated control	0/14	2/18 (11 %)
Distilled water	0/11	0/11
NNN (Low dose)	2/8 (25 %)	13/19 * (68 %)
NNN (Hign dose)	10/16 * (62.5%)	5/10 @ (50 %)
NNK	9/16 * (56 %)	10/13 b (77 %)

Statistical significance:
* P< 0.01 as compared to untreated and distilled water treated controls.
@ P< 0.05 as compared to distilled water treated controls.
b P <0.001 as compared to untreated and distilled water treated controls.

Discussion

In the carcinogenicity studies on Swiss male mice, both NNN and NNK proved to be potent carcinogens, inducing tumors in the lungs, forestomach and liver. These findings are in agreement with those of the other workers (Hecht et

al 1984, Hoffmann et al 1985). However, no oral tumors were induced by NNN and NNK.

The induction of lung tumors is in agreement with all the previous reports on the tumorigenicity of NNN and NNK, wherein, respiratory tumors were induced in all the experiments irrespective of the route of administration (International Agency For Research on Cancer 1985). The induction of forestomach papillomas as a result of treatment with various nitrosamines have been reported earlier (Emminger and Mohr 1982, Mirvish et al 1983). Hirsch et al (1986) have reported the observation of papillary hyperplasia of the forestomach in rats treated with snuff.

Present study, thus proves that NNN and NNK are strong carcinogens, inducing tumors in the lung, forestomach and liver of Swiss male mice.

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Induction of dominant lethals by TMTD in mice

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Summary

Experiments were carried out to test the mutagenic potential of TMTD (tetramethylthiuram disulfide) using dominant lethal assay. Dominant lethality represents embryonic death resulting from the chromosomal breakage in gametes of parents. TMTD induced significant increase of dominant lethals in mice treated with different doses when compared to controls.

Introduction

TMTD is mainly used in the rubber industry as an accelerator and in agriculture as a fungicide. Rubber accelerators are highly reactive chemicals used to accelerate the vulcanizing process and ensure better final properties of the product.

Studies have been carried out on the induction of mutagenic effects of TMTD. There are reports on the positive evidence for mutagenic effects in microorganisms (Hedenstedt et al 1979), plants like Aspergillus nidulans (Zdzienicka et al 1981), Drosophila melanogaster (Donner et al 1983) and mammalian cell cultures (Hinderer et al 1983). It is also reported that TMTD is teratogenic in mice (Matthiaschk 1973). In the present investigation various doses of TMTD are tested for its mutagenicity using dominant lethal assay to asses the genetic effects in mice.

Materials and methods

Swiss albino mice obtained from National Institute of Nutrition, Hyderabad were used as test animals. The animals were caged individually at room temperature. Diet and water were provided ad libitum.

Male mice aged 7-8 weeks with body weight ranging between 23 and 24 g were used. For each dose group 10 males were treated and each male was mated with two virgin untreated females for a period of 8 weeks. The females were changed at weekly intervals.

TABLE 1 TOTAL IMPLANTATIONS PER PREGNANT FEMALE MATED TO MALE MICE TREATED WITH TMTD.

Weeks	Control I				Control II				80 mg/kg				200 mg/kg				320 mg/kg			
	Preg. Fem. No.	%	T.I	T.I / Fem.	Preg. Fem. No.	%	T.I	T.I / Fem.	Preg Fem. No.	%	T.I,	T.I. / Fem.	Preg. Fem. No.	%	T.I.	T.I. / Fem.	Preg. Fem. No.	%	T.I.	T.I / Fem
1	14	70	135	9.64	13	65	127	9.76	14	70	136	9.71	12	60	108	9.00	16	80	132	8.25
2	13	65	128	9.84	14	70	141	10.07	16	80	152	9.50	14	70	127	9.07	13	65	123	9.46
3	16	80	143	8.93	14	70	139	9.92	12	60	120	10.00	11	55	108	9.81	17	85	142	8.35
1-3	43	71.6	406	9.47	41	68.3	407	9.91	42	70	408	9.73	37	61.6	343	9.29	46	76.6	397	8.68
4	15	75	120	8.00	16	80	159	9.93	15	75	124	8.26	16	80	142	8.87	14	70	122	8.71
5	12	60	110	9.16	11	55	107	9.72	13	65	125	9.61	13	65	122	9.38	11	55	104	9.45
4-5	27	67.5	230	8.58	27	67.5	266	9.82	28	70	249	8.93	29	72.5	264	9.12	25	62.5	226	9.08
6	15	75	121	8.06	12	60	107	8.91	11	55	102	9.27	17	85	135	7.94	12	60	103	8.58
7	14	70	139	9.92	15	75	121	8.06	14	70	129	9.21	15	75	122	8.13	13	65	124	9.53
8	14	70	134	9.57	14	70	132	9.42	12	60	106	8.83	11	55	109	9.90	10	50	79	7.90
6-8	43	71.66	394	9.18	41	68.3	360	8.79	37	61.6	337	9.10	43	71.6	366	8.65	35	58.3	306	8.67

Preg. Fe. : Pregnant females.
T.I. : Total implantations.
T.I. / F : Total implantations per female.

$P < 0.05$

TABLE 2 LIVE IMPLANTATIONS PER PREGNANT FEMALE MATED WITH MALE MICE TREATED WITH TMTD.

Weeks	Control I			Control II			80 mg/kg			200 mg/kg			320 mg/kg	
	Preg. Fem.	L.I.	L.I./Fem.	Preg. Fem.	L.I.	L.I./Fem.	Preg. Fem.	L.I.	L.I./Fem.	Preg. Fem.	L.I.	L.I./Fem.	Preg. Fem.	L.I.	L.I./Fem.
1	14	128	9.14	13	122	9.38	14	127	9.07	12	98	8.16	16	119	7.43
2	13	122	9.38	14	135	9.64	16	141	8.81	14	115	8.21	13	111	8.53
3	16	135	8.43	14	132	9.42	12	111	9.25	11	99	9.00	17	126	7.41
1-3	43	385	8.98	41	389	9.48	42	379	9.04	37	312	8.45	46	356	7.79
4	15	114	7.60	16	152	9.50	15	114	7.60	16	130	8.12	14	105	7.50
5	12	105	8.75	11	102	9.27	13	115	8.84	13	111	8.53	11	94	8.54
4-5	27	219	8.17	27	254	9.38	28	229	8.22	29	241	8.32	25	199	8.02
6	15	114	7.60	12	101	8.41	11	93	8.45	17	121	7.11	12	90	7.50
7	14	134	9.57	15	116	7.73	14	120	8.57	15	110	7.33	13	111	8.53
8	14	128	9.14	14	127	9.07	12	98	8.16	11	101	9.18	10	70	7.00
6-8	43	376	8.77	41	344	8.40	37	311	8.39	43	332	7.87	35	271	7.67

Preg. Fem. : Pregnant females., $P < 0.05$

L.I. : Live implantations

$\frac{\text{L.I.}}{\text{Fem.}}$: Live implantations per female.

TABLE 3 DEAD IMPLANTATIONS PER PREGNANT FEMALE MATED TO MALE MICE TREATED WITH TMTD.

	Control I				Control II				80 mg/kg				200 mg/kg				320 mg/kg			
Weeks	Preg. Fem	D.I.	D.I. / Fem	D.I. (%)	Preg. Fem	D.I.	D.I. / Fem	D.I. (%)	Preg. Fem	D.I.	D.I. / Fem	D.I. (%)	Preg. Fem	D.I.	D.I. / Fem	D.I. (%)	Preg. Fem	D.I.	D.I. / Fem	D.I. (%)
1	14	7	0.50	5.18	13	5	0.38	3.93	14	9	0.64	6.61	12	10	0.83	9.25	16	13	0.81	9.84
2	13	6	0.46	4.68	14	6	0.42	4.25	16	11	0.68	7.23	14	12	0.85	9.44	13	12	0.92	9.75
3	16	8	0.50	5.59	14	7	0.50	5.03	12	9	0.75	7.50	11	9	0.81	8.33	17	16	0.94	11.26
1-3	43	21	0.48	5.15	41	18	0.43	4.40	42	29	0.69	7.11*	37	31	0.83	9.00*	46	41	0.89	10.28*
4	15	6	0.40	5.00	16	7	0.43	4.40	15	10	0.66	8.06	16	12	0.75	8.45	14	17	1.21	13.93
5	12	5	0.41	4.54	11	5	0.45	4.67	13	10	0.76	8.00	13	11	0.84	9.01	11	10	0.90	9.61
4-5	27	11	0.40	4.77	27	12	0.44	4.53	28	20	0.71	8.03*	29	23	0.79	8.73*	25	27	1.05	11.77*
6	15	7	0.46	5.78	12	6	0.50	5.60	11	9	0.81	8.82	17	14	0.82	10.3	12	13	1.08	12.62
7	14	5	0.35	3.59	15	5	0.33	4.13	14	9	0.64	6.97	15	12	0.80	9.83	13	14	1.07	11.29
8	14	6	0.42	4.47	14	5	0.35	3.78	12	8	0.66	7.54*	11	8	0.72	7.33	10	9	0.90	11.39
6-8	43	18	0.41	4.61	41	16	0.39	4.50	37	26	0.70	7.77	43	34	0.78	9.05*	35	36	1.01	11.76*

Preg. Fem. : Pregnant females

D.I. : Dead implantations.

D.I. / Fem. : Dead implantations per female.

D.I. (%) : Percentage of dead implantations.

* $P > 0.05$

Three different concentrations of the chemical, TMTD (80, 200 and 320 mg/kg) were used. Since TMTD was not soluble in water it was suspended in 5% gum acacia. Doses were selected considering the LD_{50} value of TMTD for oral administration.

The treated male mice were mated sequentially with groups of untreated virgin females. Matings in weeks 1 to 4 and 5 to 8 after treatment of male mice represent samples of post-meiotic and pre-meiotic stages of spermatogenesis respectively. The females were replaced by fresh virgins at weekly intervals. This sequential changing of females were continued for 8 weeks to see the mutagenic effect on the complete spermatogenic cycle. Appropriate controls were maintained. The mice exhibiting vaginal plugs were sacrificed on the 14th day of gestation by cervical dislocation to expose the uterine contents. The abdominal cavity was cut open midventrally and the number of live and dead implantations were recorded. The dominant lethals represented the total of pre and post-implantation losses. The post implantation lethality was determined in terms of dead implants per pregnant female (Epstein and Rohrborn 1971) and total losses were calculated based on the live implantations per pregnant female.

Results and Discussion

Results were analysed weekwise corresponding to post-meiotic (1-3), meiotic (4-5) and pre-meiotic stages (6-8). The data on total implantation rates, live implantations and dead implantations in controls and in treated groups are presented in Tables 1- 3. The frequency of dead embryos was increased in treated groups when compared to controls. Pregnancy rates and post implantation losses between controls and treated groups were tested for statistical significance by using 2x2 contingency test. There was no significant difference in pregnancy rates between control and treated groups. Post implantation losses were significant in treated groups when compared to controls. Total and live implantations per pregnant female between the control and treated animals were analysed using t-test. There was no significant difference in total and live implantations per pregnant female between the control and treated groups.

Earlier we have reported a significant increase in the frequency of abnormal sperms and chromosomal aberrations in germ cells of mice treated with TMTD (Hema et al 1987). Similar observations were made by Zdzienicka et al (1982) who reported significant increase in abnormal sperms in male mice. TMTD has proved to be a relatively embryotoxic agent (Jelinek and Rychter 1980). Korhonen et al (1982) reported that TMTD, TMTM and TETD produced malformations and deaths in chicken embryos. It is observed from this study that TMTD causes

chromosome breaks in post-meiotic, meiotic and pre-meiotic stages of male germ cells resulting in intrauterine deaths of embryos.

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Prevention of the anaphylactoid reaction - the mutated gene manifestation of Tween 20 in dogs by cimetidine

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Summary

It is reported that Tween $_{20}$ - the polyoxyethylene sorbitan monolaurate could produce anaphylactoid reaction only in the canis genus viz., dogs, foxes, wolves, etc., and not in any other genus of vertebrata viz., rats, rabbits, monkeys, man, etc., by interacting with the T_{20} receptor protein-the resultant of the mutated autosomal recessive gene manifestation with threshold effects during the course of evolution in genus canis. Our aim is to block this genetically determined anaphylactoid reaction-manifested by skin flushing, scratching, vomiting and hypotension.

It is demonstrated that Tween$_{20}$ produce anaphylactoid reaction in dogs by stimulating the release of histamine like autacoid into circulation from mast cells. The promethazine - H_1 receptor antagonist (manifestations of anaphylactoid reaction is mediated through H_1 receptors) failed to prevent the hypotension due to Tween$_{20}$ whereas it prevented the hypotension due to compound 48/80 and histamine. The H_2 receptor antagonist-cimetidine completely prevented the anaphylactoid reaction in dogs whereas other H_2 receptor antagonists like metiamide failed to do so. The possible mechanisms how the cimetidine prevented the anaphylactoid reaction and the mutated autosomal recessive gene manifestation of Tween$_{20}$ in dogs are discussed in detail in the paper.

Introduction

Tween$_{20}$ - the polyoxyethylene sorbitan monolaurate is a surface active agent and dissolves various membranes in high concentration. It is observed that neither allergic nor depressor response to Tween$_{20}$ in the rat, cat, guniea pig, rabbit, monkey, chicken or man (Krantz etal 1948). But it is reported that Tween$_{20}$ produced anphylactoid reaction not only in the species of familaris (dogs) of the genus canis but also in the other species of the same genus viz., gray foxes, timber wolves, coyotes, jackals but not in any other genera of the family camidae(Krantz etal 1949).

The anaphylactoid reaction due to $Tween_{20}$, in dogs is characterized by skin flushing, scratching, vomiting and the prolonged fall of blood pressure which may be due to the release of histamine or histamine like autacoid into circulation (Krantz etal 1949). $Tween_{20}$ stimulates the receptor protein of skin mast cells which is the resultant of the mutated autosomal recessive gene. The mutation in the autosomal recessive gene might have occurred during the course of evolution and still existed due to natural selection (Goth 1967).

The fall in the blood pressure is the most characteristic in canine anaphylaxis (Goth 1967). Our aim is to prevent this genetically determined anaphylactoid reaction in dogs by using pharmacological tools.

Materials and methods

Dogs of either sex (Visakhapatnam street dogs-mongrel type) weighing 8-12 kgs. are anaesthetised with phenobarbitone sodium (180 mg/kg in saline). The carotid artery is cannulated and connected to haemodynamic set up to record the blood pressure of the animal on kymograph.

The plasma, taken from dogs in maximal hypotensive state after infusion of $Tween_{20}$(5mg/kg) is deproteinised with 8% perchloric acid and the precipitate is removed by centrifugation (2000 rpm for 10 minutes). The supernatant is deprotieinised plasma of $Tween_{20}$($DP\text{-}T_{20}$).

Chemicals used are promethazine. HCl(Phenergan, May and Baker Ltd., Bombay), $Tween_{20}$ (SD chemicals, Bombay 5% v/v solution in saline) Histamine acid phosphate(BDH, England), compound 48/80 (Sigma USA) metiamide and cimetidine (SK and F, Carolina).

Experiments

1. Promethazine is injected into circulation of dog at the dose of 1.5 kg - produced hypotension of its own. After the recovery from hypotension, histamine (1 ug/kg) compound 48/80(0.2 mg/kg) and $Tween_{20}$(1 mg/kg) are injected in presence of promethazine.

2. Cimetidine at the dose of 6 mg/kg injected into circulation of dog produced transient hypotension of its own. After restoration from lowered blood pressure, the activity of histamine (1 ug/kg) $DP\text{-}T_{20}$(4ml) and $Tween_{20}$ (ingraded doses 1 mg, 2mg, 4mg/kg) is studied.

3. Metiamide at the dose of 6 mg/kg injected into circulation of dog. It produced hypotension of its own. After recovery from hypotension, $Tween_{20}$ (1 mg/ 1kg) activity is observed.

Results

Promethazine-the H_1 antagonist has blocked the hypotension of injected histamine. The hypotensive action of compound 48/80 is partly blocked by promethazine. Promethazine failed to inhibit the hypotensive action of $Tween_{20}$. Cimetidine, the H_2 antagonist did not block the action of injected histamine completely. It also failed to block the hypotension due to compound 48/80. Cimetidine also failed to block the hypotension due to DP-T_{20}. However cimetidine completely prevented the hypotension due to $Tween_{20}$ but metiamide failed to do so.

Discussion

It is a well known fact that histamine induced hypotension in dogs is mediated by both H_1 and H_2 receptors. In our laboratory promethazine, the H_1 antagonist blocked the hypotension due to lower doses of histamine where as cimetidine, the H_2 antagonist failed to do so. Promethazine also blocked partly the hypotension due to compound 48/80 the histamine releaser where as cimetidine failed to block even to that extent. Therefore, it may be true that the hypotension due to histamine is more connected with the stimulation of H_1 receptors rather than H_2 receptors. It also appears that cimetidine is not having any effect on the release of histamine due to compound 48/80. Hence no inhibitory action on the hypotension to compound 48/80. But cimetidine completely blocked the hypotension due to $Tween_{20}$ whereas metiamide-H_2 antagonist failed to block hypotension due to $Tween_{20}$. So, it is not fair to assign H_2 antagonistic activity to cimetidine in blocking $Tween_{20}$ induced hypotension.

It is also important to note that cimetidine failed to block the hypotension due to DP-T_{20}-the deproteinised plasma of dogs, rendered hypotensive with $Tween_{20}$. Therefore, it appears that cimetidine blocked the $Tween_{20}$ action by preventing the release of autacoid into circulation of dog and it also appears that the autacoid is not likely histamine.

Cimetidine prevented completely the anaphylactoid reaction the mutated gene manifestation of $Tween_{20}$ in dogs.

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Cytogenetic effects of 2 nitro-para-phenylenediamine (2NPPD) on human leucocytes in culture

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Summary

2 nitro-para-phenylenediamine is a component of semipermanent and permanent hair dye formulations. Genotoxicity of this chemical was assessed in human lymphocytes in culture. Four different doses (25, 50, 75 and 100 ug/ml) of the chemical were tested employing the procedures for chromosome aberration analysis and sister chromatid exchanges. The chemical exhibited genotoxic effect. It increased the frequencies of chromosomal anomalies and sister chromatid exchanges at doses of 25, 50 and 75 ug/ml and at 100mg/ml, there was cell death.

Introduction

The aromatic amines are extensively employed as components of various dyes including hair colourants (IARC, 1978). These compounds are metabolically related to the nitroaromatics found in diesel exhausts and cigarette smoke. Some of them are reported to be potent mutagens (Klopman and Rosenkranz 1984) and therefore may be injurious to human health. An increased incidence of bladder cancer was reported among the workers of the dye industry (Wynder et al 1963, Anthony and Thomas 1970). 2 nitro-p-phenylenediamine (2NPPD) forms an important constituent of semipermanent and permanent hair dye formulations. It also finds application in fur dyeing and produces a red-brown colour.

There have been a few attempts to elucidate the genotoxicity of this chemical. 2NPPD was shown to be a potent mutagen in Salmonella typhimurium TA 1538 (Ames et al 1975, Searle et al 1975, Yoshikawa et al 1975, Venitt and Searle 1976, Garner and Nutman 1977, Ammenheuser and Warren 1979). It was found to be a direct acting mutagen in Salmonella typhimurium TA 98 and TA 100. The strain TA 98 proved to be more sensitive in the presence of S_9 (Gentile et al 1987). This chemical was reported to be clastogenic in human lymphocytes in culture (Searle et al 1975), in Chinese hamster prostate cells (Kirkland and Venitt 1976) and in Drosophila melanogaster it was able to induce lethality and chromosome damage (Leethem et al 1985). It induces unscheduled DNA synthesis in Hela

cells (Martin et al 1978) and is shown to be weakly positive in the hepatocyte primary culture for DNA repair (Willians et al 1982). In percutaneous absorption studies, ^{14}C labelled 2NPPD was shown to have an intermediate degree of skin penetration in human, monkey and mice (Marzulli et al 1981). Carcinogenic potential of 2NPPD was shown employing cell transformation assay in C3H/ 1OT1/2 mouse cells (Benedict 1976) and it is reported to induce liver tumours in the female mice (NCI/Bioassay 1979). On the other hand it has been reported by Adam (1985) that there was no increase in the incidence of metaphases with aberrations in the bone marrow and Ehrlich ascites tumour cells of mice invivo after treatment with 2NPPD.

A perusal of the available reports suggests the need to carry out a detailed study on the genotoxicity of 2NPPD on human leucocytes in culture. The present study is an attempt in this direction. The frequencies of chromosomal aberrations and sister chromatid exchanges induced by the chemical in cultured human lymphocytes and its effect on cell kinetics and mitotic index have been assessed.

Materials and methods

2 nitro-p-phenylenediamine (M/s. E. Merck) was dissovled in 10% dimethylsulphoxide. Its chemical formula is $C_6H_7N_3O_2$ and it has the molecular weight 153.16.

Lymphocyte cultures : Leucocytes from the peripheral blood of a healthy individual were cultured according to the method of Hungerford (1965). O.5 ml of heparinised blood was inoculated into culture vials containing 5 ml of McCoys 5a medium, 1.0 ml. of AB serum and 0.2 ml. of phytohemagglutinin. For the determination of SCE a modified method of Perry and Wolff (1974) was followed and the amount of Bromodeoxyuridine (Sigma) added to the culture was 5 ug/ml. Cultures were exposed to 25, 50, 75 and 100 ug of 2 NPPD in 10% DMSO per ml of the medium at the time of initiation. Control set of cultures were exposed to the solvent alone.

The cultures were harvested at 48h for chromosomal aberrations and at 72h for the study of sister chromatid exchanges. A minimum of 200 metaphases per experimental point were analysed. Aberrations were classified as per the report of Buckton and Evans (1982). Mitotic indices were scored for each experimental point from 5000 cells. Frequencies of SCEs were computed by analysing 25 well differentiated second division metaphases per dose point. Analysis of 200 consecutive metaphases provided data on cell kinetics.

TABLE 1 FREQUENCIES OF CHROMOSOMAL ABERRATIONS OBSERVED IN LEUCOCYTES IN CULTURE EXPOSED TO 2NPPD

Treatment	No. of Metap-hases Exam	Structural Aberrations G'	G"	B'	B"	F	No. of Aberrant Meta-phases	% of Aberrant Meta-phases	% of Aberrations + Gaps	% of Aberrations - Gaps	Mitotic index (%)
UNTREATED	200	2	-	-	-	-	2	1.0	1.0	0	1.92
SOLVENT CONTROL (DMSO 10%)	200	1	-	1	-	-	2	1.0	1.0	0.5	1.28
2NPPD											
25 ug/ml.	200	2	-	1	4	3	10	5.0	5.0*	4.0*	0.64
50 ug/ml.	200	5	-	6	2	2	15	7.5	7.5*	5.0*	0.34
75 ug/ml.	200	8	4	7	2	2	20	10	11.5*	5.5*	0.29
100 ug/ml.	NIL	CELL DEATH									0

* Significant with 'Z' test
Period of exposure : 48 h.

Results

a. Mitotic Index : There was a dose dependent decrease in the mitotic index (Table 1). At 25 ug/ml 2 NPPD decreased the cell proliferation by 50% (0.64%) compared to the solvent control (1.28%). At doses of 50 and 75 ug/ml further inhibition of cell proliferation (0.34% and 0.29% respectively) was seen.

b. Clastogenic effects : Exposure of cells to various concentrations (25, 50 and 75 ug ml) of 2NPPD for a duration of 48h resulted in a significant proportion of aberrant metaphases (Table 1). Increase was observed even when gaps were not included amongst the types of aberrations. In addition to gaps, other types of chromosomal damage observed were chromatid and isochromatid breaks and fragments. Exchange figure was not observed. There was cell death at 100 ug/ml the highest dose employed.

c. SCE and Cell Kinetics : Chronic exposure to 2NPPD (at 25,50 and 75 ug/ml) induced a significant dose-dependent increase in the frequency of SCEs (Table 2). 2NPPD inhibited cell proliferation when present throughout the culture period (0-72h). At 50 and 75 ug of 2NPPD per ml, there was inhibition of proliferation as shown by the decline in the proportion of third cycle metaphases, simultaneous with a linear increase, in the proportion of first cycle metaphases (Table 3).

TABLE 2 FREQUENCIES OF SISTER CHROMATID EXCHANGES OBSERVED IN CULTURED LEUCOCYTES EXPOSED TO 2NPPD.

Treatment	Cells Analysed (n)	SCE's (n)	Mean	Range
Untreated	25	145	5.80	2 - 10
Solvent control (10% DMSO)	25	172	6.85	4 - 14
2NPPD				
25 ug/ml.	25	296	11.84	6 - 20
50 ug/ml.	25	356	14.24	9 - 25
75 ug/ml.	25	386	15.44	8 - 26

TABLE 3 DATA ON CELL KINETICS AS COMPUTED FROM SISTER CHROMATID EXCHANGES

Treatment	First cycle	Second cycle	Third cycle
UNTREATED	25.6	57.6	16.8
Solvent control (10% DMSO)	41.0	57.5	1.5
2NPPD 25 ug/ml.	43.6	47.0	9.4
50 ug/ml.	80.0	15.5	4.5
75 ug/ml.	87.6	12.4	—

Discussion

Of the invitro assays, leucocytes or cultured mammalian cell lines are considered most suitable for the screening of mutagens (Preston et al 1981). Most mutagens are known to induce chromosomal aberrations (Natarajan and Obe 1982) and most potent inducers of SCEs are found to be S-dependent (Natarajan and Obe 1986). Assessment of SCE is a highly sensitive test in the evaluation of the mutagenic activity of some chemicals, more sensitive than that of chromosomal aberrations.

In the present study 2NPPD appears to be a clastogen. This is indicated by the significant induction of aberrant metaphases in cultures treated with the chemical. The predominant type of aberrations include chromatid gaps and breaks probably induced in S or G phase of the cell cycle. According to Bender et al (1974) it is the unreplicated, incomplete or misrepaired DNA lesions that appear as breaks and gaps in the ensuing mitosis. Reports are available on the clastogenicity of 2NPPD in human lymphocytes (Searle et al 1975), in cultured chinese hamster cells (Kirkland and Venitt 1976) and in Drosophila melanogaster (Laethem et al 1985).

The percentage of chromosomal aberrations (with or without gaps) seems to be dose dependent, so also the reduction in mitotic index (MI) which finally leads to cell death at the highest dose tested, namely 100 ug/ml. This reduction in MI is in agreement with the observation of Smith et al (1976) who reported depression of lymphocyte transformation by 2NPPD. Increase in the percentage of chromosomal aberrations was seen with and without gaps. The percentage of gaps induced by a test chemical may also be dose dependent (Gebhart 1977) and therefore may be a good indicator of clastogenicity as any other chromosomal aberrations (Anderson and Richardson 1981). These authors suggested that if gaps are artifacts of staining and processing, the control cultures also should present a similar incidence. In the present study gaps did not show a higher frequency in the control set. Therefore gaps may be considered as sensitive indicators of chromosome damage and may be included in the statistical evaluation of the data. There was a significant increase in the incidence of SCEs over the base line as revealed by a doubling effect in cultures exposed to 50 and 75 ug/ml. of 2NPPD. This increase in the mean frequency of SCE also appears to be dose-dependent. The results obtained in the present study indicate a positive correlation between the two end points, namely chromosomal aberrations and SCEs which are known to register clastogenicity and mutagenicity respectively. 2NPPD therefore can be considered to act as a direct mutagen. It was also found to be direct mutagen in Salmonella typhimurium TA 98 and TA 100 (Gentile et al 1987). This was found to be increased further in the presence of S9 when tested employing the strain TA 98.

2NPPD is an aniline derivative with a nitro-group. It was observed by Sontag (1981) that the aniline derivatives are biologically least active when the amino groups are para to one another. On the other hand the activity is increased as the amino groups become ortho to the substituted group. These compounds containing a nitro group are better direct acting mutagens than those without a nitro group (Milman and Peterson 1984).

A study of the cell kinetics reveals that 2NPPD causes retardation in the progress of the cell cycle with an accumulation of cells in the first cycle. This is in agreement with the observation of Smith et al (1976) that 2NPPD was more potent inhibitor of blastic transformation than 4NOPD. Reduction in mitotic index as observed in the present study finds further support in the observations on the delay in the progression of cell cylcle recorded in Table 3.

Results thus suggest that 2NPPD is genotoxic in cultured lymphocytes. Whether mammals in vivo respond similarly when exposed to the chemical, needs to be examined.

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In vitro effect of malathion on human lymphocytes

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Summary

Malathion is an organophosphorus pesticide which is extensively used in agriculture. It was tested on peripheral lymphocytes of healthy males in culture at four different concentrations (0.375, 0.75, 1.5 and 3 ug/ml)and three different durations i.e, 24 h, 48 h and 72 h. There was a dose dependent increase in chromosomal aberrations but it was significant only at 3ug concentration. A significant increase in sister chromatid exchanges (SCEs) was observed with increased concentration and duration of exposure.

Introduction

Malathion is one of the important organophosphorus pesticide widely used in agriculture to control pests. Cytogenetic and genetic effects of malathion have been studied ranging from bacteria to mammals. Negative results were reported in microorganisms using malathion (McCann et al. 1975; Wild 1975; Dean 1972). Malathion failed to induce chromosomal aberrations in human lymphoid cell lines and mouse bone marrow cells (Huang 1973.) Yoder et al. (1973) reported chromosomal aberrations in population exposed to different pesticides including malathion. Malathion induced SCEs in mammalian cell cultures (Nicholas et al. 1979, Chen et al. 1981, Sobti et al. 1982), but the mutagenic potential of malathion is still contradictory.

In the present study an attempt has been made to evaluate the effect of malathion on the incidence of chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes of three healthy males in culture.

Materials and methods

Intravenous blood was collected from three different donors (non smoking healthy males, age 20, 29 and 33 years). Heparinized whole blood was grown in RPMI 1640 medium supplemented with 25% human AB serum, 0.5% phytohemagglutinin and 0.25% antibiotic dicristicin. Malathion was obtained

TABLE 1 FREQUENCY OF CHROMOSOMAL ABBERRATIONS IN HUMAN LYMPHOCYTES TREATED WITH MALATHION AT 24, 48 and 72 h

Treatment ug/ml culture	No. of meta-phases scored	Chromatid				Total No. of aberrations without gaps ±S. D.
		Gaps	Breaks	Fragments	Deletions	
24 Hrs:						
Control I	300	4(1.33)	2(0.67)	0.0	0.0	2 ± 0.58 (0.67)
Control II	300	4(1.33)	2(0.67)	0.0	0.0	2 ± 0.58 (0.67)
0.375	300	4(1.33)	1(0.33)	0.0	0.0	1 ± 0.58 (0.33)
0.75	300	5(1.67)	4(1.33)	0.0	0.0	4 ± 1.00 (1.33)
1.5	300	6(2.00)	5(1.67)	0.0	0.0	5 ± 1.00 (1.67)
3.0	300	5(1.67)	5(1.67)	1(0.33)	0.0	6 ± 1.00 (2.00)
48 Hrs:						
Control I	300	4(1.33)	2(0.67)	0.0	0.0	2 ± 0.58 (0.67)
Control II	300	4(1.33)	2(0.67)	0.0	0.0	2 ± 0.58 (0.67)
0.375	300	5(1.67)	3(1.00)	0.0	0.0	3 ± 1.00 (1.00)
0.75	300	5(1.67)	6(2.00)	0.0	0.0	6 ± 1.00 (2.00)
1.5	300	6(2.00)	7(2.33)	0.0	0.0	7 ± 0.58 (2.33)
3.0	300	7(2.33)	7(2.33)	2(0.67)	0.0	9 ± 1.00 (3.00)*
72 Hrs:						
Control I	300	4(1.33)	2(0.67)	0.0	0.0	2 ± 0.58 (0.67)
Control II	300	5(1.67)	2(0.67)	0.0	0.0	2 ± 0.58 (0.67)
0.375	300	5(1.67)	4(1.33)	0.0	0.0	4 ± 0.58 (1.33)
0.75	300	7(2.33)	6(2.00)	0.0	0.0	6 ± 1.00 (2.00)
1.5	300	7(2.33)	7(2.33)	0.0	0.0	7 ± 0.58 (2.33)
3.0	300	9(3.00)	7(2.33)	2(0.67)	1(0.33)	10 ± 1.53 (3.33)*

* $P < 0.05$, values given in parentheses are percentages, @ Standard deviation for three donors.
Control I given an equal volume of distilled water, Control II given an equal volume of 1% DMSO.

TABLE 2 FREQUENCY OF SISTER CHROMATID EXCHANGES IN HUMAN LYMPOCYTES TREATED WITH MALATHION At 24, 48, 72 h.

Treatment ug/ml. medium	No. of meta-phases scored	24 h. treatment SCE/cell ± s.e.m.	48 h. treatment SCE/cell ± s.e.m.	72 h. treatment SCE/ cell ± s.e.m
Control I	150	3.70 ± 0.57	3.84 ± 0.60	4.08 ± 0.67
Control II	150	3.48 ± 0.30	3.92 ± 0.63	3.91 ± 0.71
0.375	150	5.42 ± 0.27*	4.70 ± 0.50*	5.13 ± 0.37*
0.75	150	5.62 ± 0.43*	6.00 ± 0.90*	6.37 ± 0.74*
1.50	150	6.68 ± 0.60*	7.67 ± 0.33*	8.43 ± 0.40*
3.00	150	8.76 ± 0.82*	10.36 ± 0.57*	11.19 ± 0.63*

* $P < 0.05$

Control I (given an equal valume of distillied water).
Control II (given equal volume of 1% DMSO).

from Artee Minerals, Faridabad, Haryana. The lymphocytes were treated with 0.375, 0.75, 1.5 and 3 ug of malathion 50% per ml. culture medium for 24, 48 and 72 hrs. (chemical was added at 48 hrs, 24 hrs and 0 hrs, after initiation of the blood respectively). Since malathion was not soluble in water, it was dissolved in 1% dimethylsulfoxide (DMSO). Control cultures were maintained simultaneously with distilled water (Control I) and 1% DMSO (Control II). A separate set of cultures were treated with 3 ug/ml of bromodeoxyuridine (BrDU) at the time of initiation. All the cultures were incubated at 37°C for 72 hrs. The cultures were terminated by adding colchicine (0.1 ug/ml) and the slides were prepared according to the method described (Moorhead et al. 1960). Slides with BrDU cultures were kept in dark for three days and were processed according to the standard method of Perry and Wolff, (1974) for differential staining.

A total of 100 metaphases were scored for each donor for each concentration for chromosomal aberrations and 50 metaphases for SCEs. Since the chromatid gaps were not stable aberrations they were excluded from the total number of aberrations. Statistical analysis of the data was made using X^2 test for chromosomal aberrations and Student 't' test for SCEs. All the data were compared with control data.

Results

The data on chromosomal aberrations with different concentrations and three durations are given in table 1. There was a dose dependent increase in total chromosomal aberrations with the duration of treatment. Gaps and breaks were observed at all the concentrations whereas deletions and fragments were observed only at 3 ug concentration. Incidence of SCEs is given in table 2. There was a significant increase in SCEs at all the concentrations and at different time intervals. The data of three donors were pooled and presented in respective tables. Standard deviation has been given for total chromosomal aberrations.

Discussion

Total chromosomal aberrations were significantly high at 3 ug/ ml concentraion. Absence of unstable type of aberrations (dicentrics and rings) and presence of chromatid gaps, breaks, deletions and fragments at highest concentration indicated that chemical acts mostly on synthetic phase of the cell cycle. Similar observations were reported by Walters et al. (1980). Induction of micronuclei with malathion was reported in mice (Dulout et al. 1982,Salvadori et al. 1988). Chromosomal aberrations were increased in population exposed to pesticides including malathion (Rupa et al. 1988, Rita et al. 1987, Van bao et al. 1977).

A significant increase in SCEs was observed at all the concentrations and at three time intervals. Present results are in accordence with the earlier studies, who observed a high frequency of SCEs in human lymphoid cells (Sobti et al. 1982), Chinese hamster cells(Chen et al. 1981) and in human fibroblasts (Nicholas et al. 1979).

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Cytogenetic damage induced by potassium dichromate on human leucocytes in culture

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Summary

Cytogenetic effect of potassium dichromate has been tested on human lymphocytes in culture at 4 different doses. It was found to be both cytotoxic and clastogenic in culture.

Introduction

Environmental pollution by certain metal compounds causes serious problems including genetic hazards in man. Chromium is an important metal in industries. It is used for tanning in leather industries and for chrome plating (Leonard and Lauwerys, 1980). In view of its extensive use in industries and the danger of it being an occupational hazard, chromium toxicity has attracted the attention of investigators in the fields of genetics and cancer. Presence of large amounts of chromium was reported in the lungs, resulting in high incidence of lung cancer in workers occupationally exposed to chromium (Davies 1978, Hayes et al1979, Satoh et al 1981, Alderson et al 1981).

Besides being an occupational hazard, chromium present in industrial wastes will find its way into sewage sludges. These on disposal to agricultural lands may lead to its accumulation in plants (Shivas1980) and thus causing food contamination. Presence of high amounts of chromium in drinking water has been reported in an industrial area of Madras. A large number of industries utilizing chromium are located in this part of the country. In view of its toxicity and its association with carcinogenesis, it was thought desirable to evaluate the genotoxic effects of this element.

Materials and methods

$K_2Cr_2O_7$ (obtained from M/s. Glaxo Laboratories, India) was dissolved in double distilled sterile water. Leucocytes from peripheral blood obtained from

TABLE 1 FREQUENCIES OF CHROMOSOMAL ABERRATIONS IN CULTURED LYMPHOCYTES EXPOSED TO POTASSIUM DICHROMATE

Treatment	Total No. of Cells Analysed	Total No. aberrant Cells		Types of Aberrations						Total No. of Aberrations			Abberations Per Cell		
				*D	G	B	F	EF	M	With Gaps		Without Gaps	With Gaps		Without Gaps
		n	%							n	%	n	%		
Control	3725	124	3.33	—	106	19	-	-	-	125	3.35	19	0.51	0.0335	0.0051
$0.5x10^{-6}M$	1000	96	9.6	—	78	21	1	-	-	100	10	22	2.2	0.1	0.022
$1x10^{-6}M$	1000	132	13.2	1	102	34	1	1	-	139	13.9	37	3.7	0.139	0.037
$2x10^{-6}M$	1000	163	16.3	—	143	34	2	1	1	181	18.1	38	3.8	0.181	0.038
$4x10^{-6}M$	1216	154	12.66	4	120	38	2	-	-	164	13.49	44	3.62	0.135	0.0362

* D = Deletion G = Gap B = Break F = Fragment EF = Exchange Figure M = Minute.

healthy individuals were cultured at 37°C as per the procedure of Hungerford (1965). 24 hours after the initiation of culture, $K_2Cr_2O_7$ was added. Four different doses (4×10^{-6}, 2×10^{-6}, 1×10^{-6} and 0.5×10^{-6} M) were tested. Chromosome preparations were obtained from 72 h cultures as per the standard air drying method (Hungerford 1965). 100 well spread metaphases were analysed from each culture. Results obtained were subjected to statistical analysis using Chi-square test.

TABLE 2 MITOTIC INDICES OBSERVED IN CULTURED LYMPHOCYTES EXPOSED TO POTASSIUM DICHROMATE

Treatment	No. of undividing cells scored	No. of cells in division	Mitotic Index (%)
Control	187648	3748	1.96
0.5×10^{-6}M	50115	1062	2.075
1×10^{-6}M	50171	901	1.764
2×10^{-6}M	50261	807	1.58
4×10^{-6}M	50976	801	1.55

Results and Discussion

Potassium dichromate is found to induce chromosomal aberrations in human lymphocytes in culture. The frequencies observed are recorded in Table 1. The aberrations observed are classified into two groups - with gaps and without gaps. Compared to the control set, there was a significant increase in the rate of aberrations. This was observed even when gaps were not included in the total incidence of aberrations. It is evident from the data on mitotic index (Table 2), that chromium reduces the number of dividing cells. The results suggest that it is both cytotoxic and clastogenic at the doses employed.

The somatic mutation theory of carcinogenesis is now widely accepted. The mutagenicity of a test compound can be considered to be a reliable indicator of the potential carcinogenicity of the chemical. There exists correlation between the processes of mutagenesis and carcinogenesis which has been established in the case of number of chemicals (McCann et al 1975, a, b, Newbold1978.)

The mechanism of action of chromates in biological system has been investigated (Champy - Hatem 1962, Mertz 1969, Schoental 1975). In a series of publications, Levis and collaborators (1976, 1977, 1978) have discussed the action of chromates on biological system. According to their hypothesis, chromates may act on mammalian cells at two levels, either at plasma membrane level or at the intracellular level. However, a perusal of the literature indicates that there is a need to carry out a detailed study on the molecular mechanism of the mutagenesis of chromium. According to Petrilli and DeFlora (1978) the hexavalent chromium ion probably induces frame shift errors and base-pair substitution in the DNA of Salmonella.

The main alteration in DNA that is induced by hexavalent chromium in intact cells is considered to be DNA-Protein and DNA-DNA cross linking (De Flora 1984). This is brought about by chromium (III) that is derived from the intracellular reduction of chromium (VI). Similar effect is also observed when chromium (III) interacts with isolated nucleii.

Depending on its power of oxidization, chromium (VI) induces single-strand breaks in DNA of intact cells, thus causing mutagenicity.

According to De Flora (1984) the fidelity of DNA synthesis is also affected by chromium (VI) as it disturbs the balance of nucleotide pool.

From the results obtained in the present study, it is difficult, to infer on the molecular mechanism of chromium induced genotoxicity. It is therefore necessary to obtain data on the nature and interaction of hexavalent and trivalent chromium with intra and extra cellular components to elucidate the mechanism of chromate mutagenesis.

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Evaluation of mutagenic effect of meprobamate in invivo and invitro test systems

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Summary

The mutagenic effect of meprobamate was studied in invivo (mice) and invitro (human lymphocyte) test systems. Therapeutic concentrations of the drug were tested. Meprobamate has failed to cause any increase in the frequency of micronucleus, chromosomal aberrations in testicular cells and abnormal sperms in mice and chromosome aberrations in human lymphocyte cultures.

Introduction

Meprobamate is a sedative used in human medicine to control anxiety, tension and psychogenic insomnia. The hypnotic use of meprobamate in patients is as effective as the benzodiazepine group of drugs. Georgiana (1973) gave evidence for the absence of chromosomal damage in female mice treated with meprobamate. There was no increase in chromosome aberrations in the oocytes or ovarian follicles. There were no reports on the effect of meprobamate on the testicular cells of mice. The present investigation is aimed to study the mutagenic effects of meprobamate in invivo and invitro test systems using male Swiss albino mice and human lymphocyte cultures.

Materials and methods

Meprobamate obtained from Wyeth laboratories, Bombay was disolved in distilled water and various concentratioins were prepared. The therapeutic concentrations tested were 4, 12 and 20mg/kg in Swiss albino mice and as 1.0, 1.5 and 2.0mg/culture in human lymphocyte cultures. Distribution of animals and treatment : 6-8 weeks old male Swiss albino mice used in the present study were obtained from Biological Evans Ltd., Hyderabad. Each mouse weighed about 25g. Meprobamate was dissolved in distilled water and three concentrations of the drug 4, 12 and 20mg/kg were administered by gavage. Control animals of the same age group were dosed similarly with distilled water of equal volume (0.5ml). 5 animals

were used for each concentration in micronucleus test and analysis of chromosomes in spermatogonia and 8 animals per concentration were used in sperm morphology assay.

The mutagenic effect of meprobamate was studied using micronucleus test, analysis of chromosomes in spermatogonia and sperm morphology assay in mice (in vivo) and analysis of chromosomes in (in vitro) human lymphocyte chromosomes.

Micronucleus test: Schmid (1975) developed this technique for the toxicological screening of drugs and chemicals. Mice were administered orally with various doses of the drug in two instalments with an interval of 24 h. The animals were killed by cervical dislocation 6 h after the administration of the last dose. The animals were dissected and bonemarrow smears were prepared according to the standard procedure (Schmid 1975). The slides were stained and screened for the presence of micronuclei in young erythrocytes of mice. 2000 polychromatic cells and a corresponding number of normochromatic cells were screened per each animal.

Analysis of chromosomes in spermatogonia: The evaluation of chromosomal damage caused in the germ cells by drugs is more important as the progeny produced by these gametes may transmit their effects to the next generation. Evans etal (1964) devised this technique for the study of mutagenic effects of chemicals on the testicular cells of mice. The animals treated with various doses of meprobamate were sacrificed by cervical dislocation on 60th day. The animals were dissected and both the testes were removed. The testes were teased in 1.2% of trisodium citrate solution and the cells were transferred into a centrifuge tube and were incubated for 25 minutes at 37°C. The suspension was centrifuged and the superanatant was discarded. The cells were fixed by adding fixative and the slides were prepared and stained with Giemsa. Well spread metaphases were screened for various types of chromosomal aberrations like autosomal univalents, sex-univalents, polyploidy, etc. 150 metaphases were screened from each animal in both control and treated groups.

Sperm morphology assay: Wyrobeck and Bruce (1975) devised this assay to detect the mutagenic effect of chemicals on the morphology of the sperm. Various doses of the drug were administered orally to the mice and the animals were killed by cervical dislocation on 35th day after treatement. The animals were dissected and cauda epididymis was dissected out and placed in 1ml of saline. The material was teased and uniform suspension was prepared. The suspension was transferred into a test tube and stained with 10% aqueous Eosin (Y). Few drops were smeared on clean glass slides and were allowed to dry. 2000 sperms were analysed from each animal in both control and treated groups.

In vitro analysis of chromosomes in human lymphocytes: Intravenous blood was collected asceptically from healthy donors and whole blood cultures were initiated in RPMI 1640 culture medium (Hi-Media, Bombay). Lymphocytes were allowed to grow for 72h, at 37 °C. Three concentrations of meprobamate (1.0,1.5 and 2.0 mg/8ml culture) were added to the cultures and the cells were treated with the drug for 24 h, 48 h and 72 h. Control cultures were treated with an equal amount of distilled water. After 72 h,, treatment the cultures were terminated by adding colchicine (3ug/ml) to arrest the cell cycle at metaphase. The cultures were harvested and slides were prepared as per the method of Moorhead etal (1960). Slides were screened and 400 metaphases were analysed per concentration for various types of chromosome aberrations like chromatid breaks, gaps, deletions, polyploidy, etc.

Results and Discussion

The incidence of micronuclei in polychromatic erythrocytes in mice is 0.18 in controls and it is 0.29 in mice treated with 20 mg/kg. The percentages of chromosomal aberrations and spermhead abnormalities are 11.87 and 1.80 in controls and the values increased to 14.66 and 2.10 after treatment with 20 mg/kg. of meprobamate respectively. The freque;ncy of micronucei, chromosomal aberrations in spermatocytes and the abnormal sperms in mice after treatment with 4, 12 and 20 mg/kg.of meprobomate did not show a significant increase when compared to the control values.

The percentage of chromatid type of aberrations in human lymphocyte cultures (in vitro) exposed to 1.00, 1.50 or 2.00 mg/kg culture also did not vary from control value. While the percentage of aberrations is 1.50 in controls it has increassed to 1.75, 2.00 and 2.00 with 1.0, 1.5 and 2.0 mg/culture of meprobomate respectively after exposure to 72 h. A similar phenomenon is obtained at 24 and 48 h. The increase was not statistically significant at any dose level at any time interval.

The data obtained from in vivo and in vitro experiments reveal that meprobomate is not mutagenic in test systems understudy. The results obtained in the present study are in accordance with the observations made by Georgiana (1973) who reported lack of evidence for induced chromosome aberrations either in oocytes or ovarian follicles Kamada et al (1971) also showed that meprobamate was not capable of inducing chromosomal aberrations in blast transformed cells of human lymphocytes.

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Evaluation of mutagenic activity of chromium in workers of chromium industries

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Summary

Occupational exposure represents the main source of human contamination by chromium. Many epidemiological surveys carried out in different countries have revealed that workers in various chromium industries have an increased risk of developing lung cancer. In the above studies a wide range of latency period varying from 10 to 27 years have been reported. Chromium exposure in industry over prolonged period is likely to influence the growth and multiplication of cells in the body i.e. the genetic mechanism of cells. These genetic effects are likely to be reflected in the chromosomes of the worker. This provides the hypothesis, for investigation of chromosomal compliment of 53 industrial workers employed in chromium based industries. These workers were also subjected to detailed health examination for assessment of their health status.

Results of the study indicate that it is possible to identify high risk workers by quantitation of cytogenetic damage. This cytogenetic study also furnishes some explanations for the higher incidence of lung cancer seen in earlier epidemiological studies.

Introduction

Chromium is a normal constituent of human body and is essential in small quantities to maintain certain normal functions. Its deficiency impairs glucose metabolism. If it exceeds a critical level it can be harmful. Since 1930, many epidemiological surveys carried out in Germany, United States and Great Britain have revealed that workers from various chromium industries have an increased risk of developing lung cancer.

Experiments to determine the carcinogenicity of chromium compounds in laboratory animals have shown that certain chromates, specially the hexavalent chromium salts, primarily those of low and medium water solubility are carcinogenic in several strains of rats and mice. Recently, electron microscopic study on

nasal mucosa of workers in a chrome paint factory had revealed precancerous changes with long term exposure to paint fumes.

In all the above studies there is a wide range of latency period, the mean latency period being 10.6 years in Mancuso's (1951) study to 27 years in a German chromate producing industry (Baetjer 1950).

Inspite of these variations and differences in reporting, there has been a consensus of opinion on the "close relationship" between occupational exposure and increased carcinogenic risk in man. And fortunately, there is a long latency period between occupational exposure and the appearance of the disease. This provides the background for the present study.

Chromium exposure over prolonged period, is likely to influence growth and multiplication of different cells in the body i.e. the genetic mechanism of cells, leading to irreversible modification of DNA replication which at a certain quantum may result in interference in growth resulting mutagenesis and carcinogenesis. These changes are likely to be reflected in the chromosomes of the worker

Materials and methods

46 chromium workers and 7 matched controls were subjected to cytogenetic analysis in addition to a detailed health examination, which included comprehensive personal and family history, clinical examination and routine laboratory investigations like haematological and urine examination. Special investigations were done as indicated to assess the health status of the workers.

DESIGN OF EXPERIMENT

Code for group		Duration of occupational exposure	Number of workers examined		Total
			Chrome Plating workers	Chrome ore grinding workers in paint factory.	
C		Control	3	4	7
Gp	I	1-5 years	6	3	9
Gp	II	6-10 years	6	13	19
Gp	III	11 years and above	14	4	18
	Total		29	24	53

For each type of industry, workers were divided into four groups. Control group included workers such as peons, watchmen, drivers etc., matched in terms of age and socio-economic background.

After the health examination, workers were assigned a category of health as follows: "A" Category of health was assigned to workers in robust health , "B" Category of health was assigned to workers who showed deviation from mental,physical or social well being.Their complaints were often vague but they were derogatory to achievement of physical, mental or social well being.
"C"Category of health was assigned to workers who are suceptible to repeated upper respiratory infections, anemia, chronic dermatitis, repeated chemical burns due to lack of attention and low resistance to diseases.

II. For assessment of genetic damage chromosomes were prepared by microculture technique. Air dried slides were stained with Giemsa stain and scanned under a high resolution microscope. Metaphases were photographed for analysis. (Fig 1-4).

III. For quantitation of genetic damage, aberrations were converted into number of breaks in each metaphase according to the following system (Hsu 1982)

ABERRATIONS	NUMBER OF BREAKS
1. Inter and Intra chromosomal bridge (per bridge)	02
2. Ring /dicentric and isochromatid fragment	02
3. Triradius/quadrilateral (per exchange point)	02
4. Tricentric	04
5. Break/fragment/terminal deletion/tip/gap	01

For purpose of quantitation, the observed damage was recorded under three headings, namely -

I. Simple breaks : unhealed aberrations like breaks, gaps, deletions, fragments were counted in each metaphase.

II. Bridges : Inter and Intra chromatid exchanges were counted in each metaphase and converted into breaks.

III. Computed damage : Unhealed aberrations, healed aberrations like rings, dicentrics, triradius etc. and bridges were added. The sum total of these, converted into breaks was also calculated.

The frequency of each type of damage under the above headings was calculated per metaphase/per worker. The data was further analysed and frequency of breaks, bridges and computed damage per metaphase per group of workers was calculated. Finally, genetic damage in each group was compared with that in the control group by applying student 't' test.

Results and Discussion

The study was planned to quantify the genetic hazard in workers of chromium based industries. An attempt was made to correlate the observed damage with the duration of occupational exposure in order to determine the limit of exposure. An attempt was also made to correlate the observed damage with the health status of the workers in order to monitor the worker's health. Analysis of the study revealed the following: i) Frequency of unhealed chromosome aberrations like chromatid breaks, gaps, terminal deletions, fragments, etc, indicated wide range of inter individual differnces. Some workers in control group exhibited a frequency of 1.16 breaks per metaphase whereas other workers indicated zero frequency. Production of breaks is only the first step of a two step process, the second step being 'Healing ' of broken arms for the chromosomes to survive and be transmitted to daughter cells. Most breaks heal by rejoining,either to restore the original configuration, or to form new chromosome rearrangements. In our opinion, presence of open breaks in metaphases of a person indicates a deficiency in genetic repair mechanism. Obviously, such an individual exhibiting high frequency of open chromosome break/damage stands a higher risk of losing damaged cells from his body due to deficient healing. Extensively damaged cells do not divide, at a certain quantum, the worker will be proned to develop anemia and low resistance resulting in repeated infections, eventually affecting his general health. At this stage he might be in 'B' category of health.

Chromatid bridges in metaphase are indicators of impending breaks in anaphase , and apparently form a good index of the impending genetic disruption. Some workers exhibited frequency of 3.33 bridges with occupational exposure of over ten years, whereas other workers exhibited a frequency of 16.15 bridges with short term occupational exposure of 2-3 years. One thing is clear from the data that workers who exhibit higher frequency of bridges with short term occupational exposure to chromium, this individual is likely to lose some genetic material in each cell cycle and this loss is eventually likely to interfere with growth and multiplication of cells.

In the present study the incidence of chromatid exchanges forming multiple chromosome clusters was very high. A number of chromosomes entangled and sticking to one another by chromatid/chromosome bridges has been called "cluster", here after.

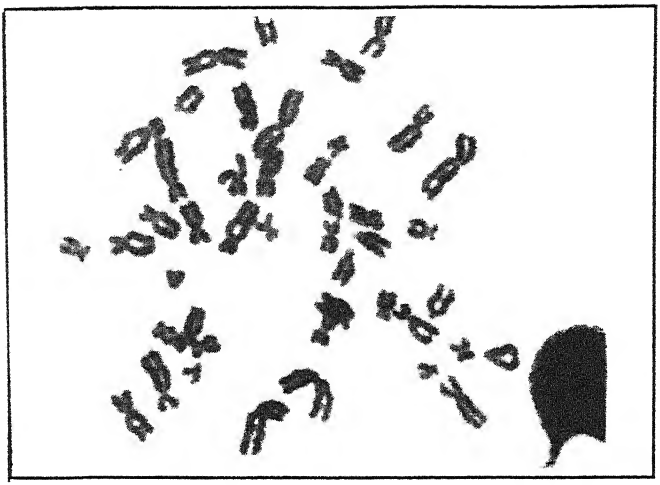

Fig - 1 A metaphase showing mild degree of interchromatid exchanges and cluster of 2 to 3 chromosomes.

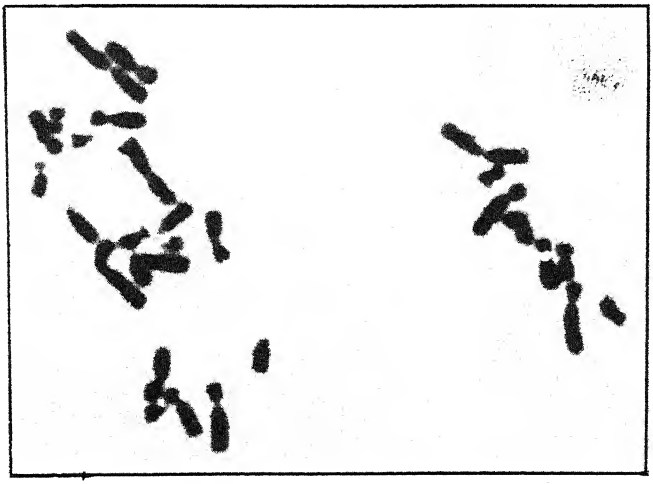

Fig - 2 A metaphase showing a good spread and five clusters. This metaphase was large and was photographed in two exposures. Each of the cluster unrelated chromosomes held close together by fine strands.

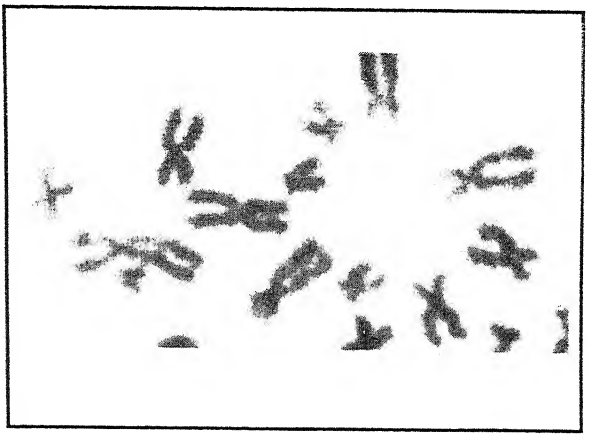

Fig - 3 Enlarged view of part of a metaphase illustrating three bridges between one G and A group chromosome, holding the two chromosomes together, as if the sides of these two chromosomes were sticking to each other. Another cluster showing four chromosomes sticking to one another at the ends. If the resolution of the microscope is not high, these fine details could easily be missed.

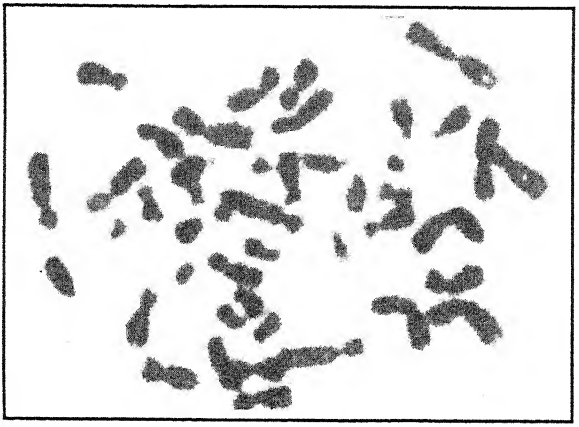

Fig - 4 A metaphase showing intense degree of interchromatid exchanges seen as chromatin strands. Almost all the chromosomes are seen to be entangled with one another. This type of metaphase could easily be overlooked in scanning or interpreted as overlap of chromosomes and might be deleted from the study by an inexperienced scorer.

Although 2 to 27 chromosmes are involved in a given cluster, the incidence of 2 to 3 chromosome cluster was most common. Multiple clustering of unrelated chromosomes was observed frequently. This phenomenon may possibly be manifestation of stickiness of unrelated chromosomes observed in mammalian cells by earlier workers. On electron microscopic studies, Manley et al (1974) postulated it to be caused by entanglement of chromatid fibres between unrelated chromosomes, as a result of abnormal condensation of chromatin prior to mitosis. There is a possibility of clustering of chromatin prior to mitosis, which being an early sign of toxicity of industrial chemicals/environmental pollutants and/or mutagens as precursor to clear chromosome damage. Such a possibility that exists as stickiness of chromosomes has been observed more frequently in cells with pathological conditions. During anaphase, the exchanges which were displayed as a chromatin bridge in metaphase eventually result in two breaks per exchange and perhaps a restitution, resulting in various degrees of chromosome damage. In the process, some genetic material may be lost. In the present study the exchanges or/ intermingling were too intense at times and scoring became too tough and time consuming. In fact metaphase with intense degree of clustering could easily be interpreted as imperfect spreads or overlap of chromosmes and these would be deleted from the study by an inexperienced scientist unless care was taken to differentiate between the two types of metaphases. Wide interindividual variation was seen in the frequency of computed damage which varied from 8 to 33.04 . The frequency of computed damage would throw light on overall damage and overall genetic mechanism which includes:-

I) Deficiency or otherwise in healing of chromosomes.
II) Impending chromatid breaks in the succeeding anaphase.
III) Union of broken segments resulting in new chromosome rearrangements.

The incidence of numerical chromosome aberrations was probably significant in workers exposed to chrome ore dust in chrome factory. Chromium ore might be affecting the spindle formation and thus may disturb the growth and multiplicaion of cells. There was no rise in numerical aberrations in chromium plating workers.

All the above types of damage are not seen in the same worker i.e., the types of damage do not coexist in the worker. It seems logical to think that each type of damage reveals a different aspect of genetic mechanism, and thus these do not coexist. These may occur singly or in different combinations.

The highest degree of genetic damage that was observed in the present study was 37 computed breaks per metaphase in a chromium plating worker with occupational exposure of 8 years. This worker was suffering from chronic anemia and repeated upper respiratory infections. This worker was assigned 'C' category

of health. The worker was advised 3 months of rest in a remote village, far from industrial pollution, high protein diet supplemented by haematinic capsules. The worker's condition improved remarkably, as revealed by the followup examination. There was a relapse after two months after resuming duty. Unfortunately alternate job could not be arranged.

This type of interference with growth and multiplication of cells of the body might lead to irreversible modification of cellular function like DNA replication which at a certain quantum may result in mutagenesis and/or carcinogenesis, which is nothing but manifestation of interference in growth and multiplication of cells.

It might be argued that a vast number of factors can cause this type of damage. True, it might not be possible to attribute the damage to chromium alone. It may be the synergistic effect of chromium and other factors like addictions, malnutrition, food additives, disease, drugs and other industrial chemicals and physical agents. But having identified an individual who stands a higher risk, itself is a big achievement. Further investigations and appropriate treatment and relief measures would help to take care of the worker's health.

Fortunately, there is a long latency period between the first appearance of the cytogenetic damage and apparent illhealth. During this period the worker may be in 'B' category of health. At this stage, his complaints may be too vague and he may not seek medical advice. If the damage is detected during the latency period, when it silently, insidiously grows in the body, without the worker being aware of it, appropriate relief measures can be provided before it is too late. It is obvious that such a worker is more prone to mutagenesis/carcinogenesis and needs periodic monitoring for increase in genetic damage and measures to arrest deterioration in health.

The present study explains, to a large extent the reasons for higher incidence of lung cancer in chromium workers, reported in epidemiological studies by earlier workers.

Acknowledgement

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Sister chromatid exchange (SCE) frequencies in tannery workers and in traffic policemen

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Summary

Sister chromatid exchange (SCE) frequencies were analysed in cultured lymphocytes from workers occupationally exposed to chromium (III) compounds in tannery and from traffic policemen who are under constant exposure to automobile exhaust pollution during their eight hours work schedule at different parts of Madras City. The mean SCE frequencies of tannery workers and traffic policemen (9.76 ± 1.34 and 13.35 ± 1.910 respectively) were high when compared with matched control group (4.42 ± 0.804) comprising men who have no history of exposure to environmental mutagens. The increase in SCE is statistically significant ($P < 0.001$) and there is a correlation between the exposure period and mean SCE frequency.

Introduction

Chromium compounds are used in dyeing, tanning industries, metal polishing and printing fabrics. The most commonly used tanning process involves the application of a tanning liquor made from basic chromium sulphate (Cr III), hence occupational exposure to chromium occurs at several distinct steps in the tannery like preparation of tanning liquors; tanning and handling of tanned leather and in fabrication of leather products. A survey of literature shows that chromium compounds are highly carcinogenic and reports revealed an increased incidence of lung cancer in chromium workers (IARC 1980). Cr(VI) compounds have been shown to induce mutation and clastogenic manifestation in different test systems and a close correlation between the carcinogenic and mutagenic effects were pointed out. Although Cr (III) compounds do not induce point mutations in bacteria (Venitt and Levy 1974, Petrilli and Deflora 1978, Nestmann et al. 1979) the available data on their clastogenic effects on mammalian cells are contradictory.

Tsuda and Kato (1977), Nakamura et al (1978), Umeda and Nishimura (1979) and Macrae et al (1979) did not find any significant increase either in chromosomal aberrations or in SCE's in mammalian cells treated with Cr(III) compounds. An increased frequency of spontaneous abortions was found in

families of workers exposed to chromium in tanning industries with respect to the unexposed population as quoted by Levis and Binachi (1982). So far no cytogenetic studies have been available on workers occupationally exposed to Cr(III) compounds in tannery.

Epidemiological studies in several countries have shown that the incidence of lung cancer is higher in urban areas than in rural (Higginson and Jenson 1977). The urban factors have been explained as due to different smoking habits,occupational and general air pollution due to variety of combustion process and automobile exhausts (ICRP, Task group on lung dynamics 1966, Wark and Warner 1976). According to Leithe (1972) the automobile exhaust generally contains NO_2, CO_2, CO,H_2, hydrocarbons, benzo (a) pyrene and lead. It is well recognised that vehicle exhaust fumes are a significant source of polycyclic aromatic hydrocarbons (PAH); the composition of which depends on factors such as types and compression of engine fuel variables and work load and presence of catalyst. In addition to microbial assay, SCE tests were carried out in in vivo and in vitro systems. Significant dose dependent increase in the SCE frequency was observed by Tong et al (1981) and de Ratt (1983) in rat liver epithelial cell lines treated with PAH compounds extracted from diesel exhausts. The induction of SCE by city smog extract was about 400 times effective than benzo (a) pyrene (Schurer et al 1980) indicating that city smog contains mutagenic substances which lead to additive or synergistic effect. Dose dependent induction of SCE and chromosomal aberrations were reported for air borne particulates in human lymphocytes in vitro (Handogny et al 1986).

Since only 30 - 40% of the organic compounds in airborne particles have been identified the contribution of unidentified compounds to the toxicological risk is significant. Therefore, the assessment of the overall mutagenic or carcinogenic activity of airpollutions or the exposed population may provide a more realistic basis for the evaluation of the possible risk for man and environment than testing individual compounds.

In order to evaluate the mutagenic potential of Cr(III) compounds in tannery and to assess the overall mutagenic potential of automobile exhaust in urban areas we have carried out the study in cultured lymphocytes of tannery workers and traffic policemen respectively for SCE.

Materials and methods

Eight workers in the age group of 21 to 30 years from a tannery located at Vaniambadi in North Arcot District of Tamilnadu, who are routinely engaged in chrome tanning were investigated to evaluate the possible mutagenic potential of Cr(III) compounds. The second group of subjects were the traffic police constables of Madras city police who were occupationally exposed to automobile exhaust pollution during their routine 8 hour schedule. The period of their service and

exposure to automobile exhaust ranged from 3 to 7 years and belong to the age group of 20 - 40 years. In addition to the above two groups, six healthy students who have no history of exposure to mutagenic chemicals and radiation were taken as controls.

Lymphocytes in whole blood were cultured in sterile RPMI-1640 medium supplemented with 10 - 15% of autologous serum, 0.1 ml of 3% L. Glutamine (BDH), 0.1 ml of (100 mg/ml) streptomycin, 0.1 ml of (100 IU/ml) Benzyl pencillin and 0.2 ml of phytohaemoagglutinin (Difco) (reconstituted). Bromodeoxyuridine (Brdu) (Sigma) at a final concentration of 10 u m was added and all cultures were incubated in the dark for 72 hours at 37°C. Three hours before the harvesting period 0.1 ml of (0.003%) colchicine was added to the culture and processed after 1 1/2 h. Chromosome preparations were made following hypotonic (0.075 M kCl) and fixative (3 : 1 methanol: acetic acid) treatment of cells. Air dried preparations aged for a day were stained for SCE by the Hoechst-Giemsa method (Perry and Wolf 1974) and SCEs were scored in atleast 20 secondary metaphases per subject on coded slides by a single observer.

Results and Discussion

A total number of 1530 SCE's were detected from 157 cells of 8 workers from tannery giving a mean SCE score of 9.76 ± 1.34 per cell and a mean value of 0.212 ± 0.028 (SD) per chromosome (Table 1).

TABLE 1 SISTER CHROMATID EXCHANGE FREQUENCIES IN TANNERY WORKERS

S.No.	Subject code	Total No. of cells	Total No. of chromosomes	Total No. of SCE	SCE / chromosomes	Mean ± SD
	CONTROL GROUP	120	5520	530	0.096	4.42 ± 0.804
1.	SCE-E-1	20	920	182	0.198	9.1 ± 3.48
2.	SCE-E-2	17	782	181	0.231	10.65 ± 3.12
3.	SCE-E-3	20	920	165	0.180	8.25 ± 3.45
4.	SCE-E-4	20	920	203	0.221	10.15 ± 3.75
5.	SCE-E-5	20	920	232	0.252	11.6 ± 3.72
6.	SCE-E-6	20	920	157	0.171	7.85 ± 2.7
7.	SCE-E-7	20	920	188	0.204	9.4 ± 2.14
8.	SCE-E-8	20	920	222	0.240	11.1 ± 4.83
		157	7222	1530	0.212	9.76 ± 1.34

From the data it is observed that the mean SCE frequency per cell ranged from 7.85 to 11.6 and the group mean value 9.76 ± 1.34 when compared to controls (4.42 ± 0.804 is found to be highly significant, ($P < 0.001$). When individual SCE data are analysed in relation to the number of years of exposure, it is found that the lowest mean SCE frequency per cell in SCE - E - 6 with an exposure period of 1.9 years at the place of work in contrast to the high SCE frequencies seen in subjects who have been exposed for more than 3 years.

It has been known that Cr(III) is less mutagenic than Cr(VI) by virtue of its inability to cross the membrane. However, it has been suggested that Cr(III) can form a complex with other molecules and these legands enter the cell and become genetically active (Warren et al 1981). Present observation of increased SCE in tannery workers may be due to this mechanism. The present findings are in contrast with those of Stella et al (1982), Majone and Rensi (1979) and Levis and Majone (1979), who reported Cr (III) compounds are ineffective in inducing SCE's in chinese hamster cell line treated with $CrCl_2$. When the data on SCE of tannery workers were analysed in relation to the duration of exposure and smoking habits, the results showed that both these factors can influence SCE frequency in a dose dependent fashion. The functional significance of increased SCE in tannery workers need to be explained. In the light of vast amount of available epidemiological and experimental data on chromium induced carcinogenicity, it could be implied that increased SCE in these workers is a precarcinogenic event in these populations. It is also possible that this mutagenic status of these workers may contribute to the fetal wastage or congenital malformation in their families as already quoted by Levis and Binachi (1982).

Data on the mean SCE frequencies in lymphocytes from traffic policemen are presented in Table 2. The mean SCE frequency per cell ranged from 10.35 ± 2.73 to 15.85 ± 4.815 with a mean value of 13.35 ± 19.10. The inter human variation for SCE level was minimum and the mean SCE frequency of this group is highly significant when compared with that of controls ($P < 0.001$) (table 3). Analysis of SCE in relation to duration of exposure to automobile exhaust pollution, although based upon a limited population size, showed occurrence of higher SCE level with number of years of exposure. It is also noted, persons with smoking habit had moderately higher levels of SCE compared to corresponding non smokers of this group. It is worth noting that subject SCE - TP 8. showing maximum SCE's in this group is found to be a heavy smoker.

Air pollutants are reported to be highly mutagenic and carcinogenic (Alfeim 1982, Pitts et al 1982, Tokiwa et al 1977, Salmone et al 1979). SCE induction has been reported already in cultured cells in vitro by some of principle compon(of automobile exhaust like polycyclic aromatic hydrocarbons (PAH), benzo pyrene and lead (Kaden et al 1973, Stella et al 1979, Salmone et al 1979, W 1979, Ohnishi et al 1980, Nordenson 1982, Clark et al 1983).

TABLE 2 SISTER CHROMATID EXCHANGE FREQUENCIES IN TRAFFIC POLICEMEN

S.NO.	Subject code	Total No. of cells	Total No. of chromosomes	Total No. of SCE's	SCE/ chromosome	Mean ± SD
	CONTROL	120	5520	530	0.096	4.42 ± 0.804
1.	SCE-TP-1	20	920	265	0.288	13.25 ± 3.985
2.	SCE-TP-2	20	920	207	0.225	10.35 ± 2.739
3.	SCE-TP-4	20	920	282	0.306	14.10 ± 4.789
4.	SCE-TP-5	20	920	288	0.313	14.40 ± 3.150
5.	SCE-TP-6	20	920	214	0.235	10.70 ± 4.340
6.	SCE-TP-8	19	874	258	0.295	13.57 ± 3.834
7.	SCE-TP-9	20	920	292	0.317	14.60 ± 2.414
8.	SCE-TP-10	20	920	317	0.344	15.85 ± 4.815
		159	7314	2123	0.290	13.35 ± 1.910

TABLE 3 MEAN FREQUENCY OF SCE IN CONTROL TANNERY WORKERS AND IN TRAFFIC POLICEMEN

Study Group	No. of subjects	SCE / CELL	
		Mean ± SE	Range
CONTROL	6	4.42 ± 0.33.	0 - 14
TANNERY WORKERS	8	9.76 ± 0.48.	0 - 20
TRAFFIC POLICEMEN	8	13.35 ± 0.675	4 - 25

* Significant at $P < 0.001$ P - by student's 't' test.

However, no in vitro studies have been made so far in man. The present study in traffic policemen who are under constant exposure to emission of automobiles showed high SCE levels irrespective of their smoking habit. It is possible that one or more of principal components of automobile exhaust could have influenced the observed induction of SCE frequency in traffic policemen.

Further work is needed to predict which component of automobile exhaust has been responsible for the increased induction of SCE in these traffic policemen. This may account for their increased risk for neoplasm involving nasopharyngeal tract, tracheal and other regions of respiratory tract.

In conclusion our study demonstrate for the first time the increased incidence of SCE's in both tannery workers and traffic policemen who are occupationally exposed to Cr (III) compounds and automobile exhaust and urban pollution respectively.

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